UNITED PLANTERS' ASSOCIATION OF SOUTHERN INDIA

TEA SCIENTIFIC DEPARTMENT

ANNUAL ADMINISTRATION REPORT

FOR

1937-38

IMPERIAL MYCOLOGICAL
28 AUG 1939
INSTITUTE

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TEA SCIENTIFIC DEPARTMENT

SCIENTIFIC STAFF

Chief Scientific Officer	..	W. S. Shaw, D.Sc., Ph.D., M.Sc., F.I.C.
Asst. Tea Scientific Officer	..	K. B. W. Jones, Ph.D., B.Sc., A.R.C.Sc., A.I.C.
Entomologist	..	S. Ananda Rau, M.Sc.
Mycologist	..	M. K. Subba Rau, B.Sc.
Soil Chemist	..	V. Jayaraman, B.A., B.Sc. (*Ag.*)

U. P. A. S. I.

TEA SCIENTIFIC DEPARTMENT

ANNUAL REPORT 1937-38.

Staff:—In pursuance of the modified policy intended for the Department, Dr. W. S. Shaw, the Chief Scientific Officer left the service of the Association on the 31st March 1938, and Dr. K. B. W. Jones, the Assistant Scientific Officer ceased duty on the 31st December 1937. Provisional notice has also been given to Mr. V. Jayaraman, the Soil Chemist that his services will not be required after the 31st March 1938. Similar notice has been served on the Field Writer.

Otherwise no changes have occurred.

Advisory Work:—Letters received during the year numbered 1,199 and the number despatched 1,164. As anticipated there was an almost complete cessation of letters from Planters after the 31st December 1937. Since there appears to be a misapprehension concerning the number of enquiries received from Planters, the following table has been drawn up in which the percentage of Planters employing the Department is shown:—

Total Number of Managers (including Messrs. James Finlay & Co.) about	170
Total Number (of at least one enquiry)	109
Percentage = 64%	

The above includes Messrs. James Finlay & Co., the managers of this Company receiving its advices from Dr. Harler. This Officer sent in 43 enquiries on all subjects to this Department during the year, and assuming one per manager, the total number of enquiries becomes 152, representing a percentage of 90% of managers sending in at least one enquiry.

If Messrs. James Finlay & Co., managers be omitted, then:—

Total number of managers	137
Total number (of at least one enquiry)	109
Percentage = 80%	

Publications:—No fresh publications have been made this year, but owing to the large demand for Dr. Jones' book, both in and out of India, a reprint of this book was considered essential and appeared in September 1937. A number of publications of the Department are now out of print, and it is unfortunate that a request for Dr. Shaw's publications by the South Indian Association in London in January 1938 could only partially be met. Reprints of the Department's publications should be made, if possible, during the coming year.

Pests and Diseases:—Pests were not in great evidence during the year, but active work by the Entomologist on the control of Helopeltis was carried out at Shendurni estate as a result of facilities placed at the Department's disposal by Mr. Murphy. The results of the investigation are given fully in the Entomologist's report.

Three fungoid diseases of an unusual nature occurred during the year. The first was Cercosporella which caused considerable denudation of bushes during the month of September; the second, termed Asterina camelliae, which is non-parasitic, was prominent in Central Travancore, whilst a third, a Phyllosticta species, affecting the new shoot of Grevillea, caused considerable alarm which was intensified by reports from Ceylon that this fungus was killing out Grevilleas in the low country. Full details of these diseases are given in the Mycologist's report.

Touring:—During the year Dr. Jones spent the month of July on Lockhart Estate in pursuance of the policy laid down by the Tea Scientific Sub-Committee that a month should be spent every year in a factory. At the request of the Central and South Travancore Planters' Associations, Dr. Shaw carried out an extensive tour during which a large number of estates were visited and discussions on various planting subjects were held with five groups of Planters. The Entomologist spent two months on Shendurni estate carrying out experiments on various insecticides as a control for Helopeltis. It is anticipated that the Entomologist will repeat his visit to South Travancore in the coming year, and it is being arranged that the Mycologist will spend an extended period in South Travancore studying the subject of 'Die back' which is becoming a prominent feature on a large number of estates.

Future Policy of the Department:—No comment is required in this direction.

Composts:—This subject is still a prominent one in South India. Everything that can be said concerning it has already been given in previous Annual Reports, and it is felt that there is no reason to alter the opinion which has been maintained by the Department. It is indeed confidently anticipated that the careful examination being undertaken by at least one Company of the costs involved in large scale compost manufacture will produce a justification for the views held by the Department.

Soils:—During the year the Soil Chemist, in addition to the analysis of composts, has been engaged in establishing methods of analysing soils chemically and mechanically. This work was intended as a preliminary to executing a soil survey of South India Tea Districts. It was proposed commencing with the Anamallais district but owing to the reorganisation of the Department, it was considered advisable to postpone this work until the new Staff has commenced its activities. In the meantime it has been considered of some utility to outline the methods employed in analytical work on the Station and these details have been given in the Appendix to this Report. The necessity for this is emphasised by the Soil Chemist standing under provisional notice.

During the last three months of this year, requests for manuring programmes for estates totalling over 7,500 acres have been received, and in most cases have been accompanied by varying numbers of soil samples. The number of soil samples at present (beginning of March) under analysis is 129.

Plant Selection:—Although no active field work has been done in this direction, attempts have been made by the Mycologist to determine the chromosome number of various tea types. This is an extremely important aspect of selection, and as the technique worked out by the Mycologist interested Dr. Tubbs, who is engaged in Ceylon on Plant selection, during his visit, it has been considered desirable to publish the work for the benefit of the tea countries occupied in

plant selection work. It appears in the Appendix to this Report, and while it is of a purely scientific nature, its inclusion serves to bring the report on the work of the Station up to date.

Co-operation:—It is gratifying to be able to report considerable progress in co-operation with other scientific workers in tea. A visit from Dr. Wellensiek, the Plant Selectionist of the Experimental Station in Java, was received in May 1937. Dr. Tubbs of the Tea Research Institute, Ceylon, visited the Station at the beginning of December 1937, and it was the result of his visit which prompted the addition to this Report of the work on chromosomes. A request from Dr. Deuss, formerly Director of the Experimental Station in Java, was also received in February 1938, but owing to the reorganisation of the Department and the fact that Dr. Deuss is particularly interested in the Chemistry of Tea and Manufacture, the question of his visit has been referred to the Tea Product Committee for disposal.

The prominence given to the previous chemical work of the Department on Theotannin and other chemical ingredients of tea in Java publications calls for particular comment. The work on Theotannin has been taken up vigorously by the scientific personnel in Java, and it is interesting to observe that various methods and conceptions discovered by the South India Department are being employed in the Java work. An interchange of correspondence and samples has also taken place to the mutual benefit of both Departments.

In Ceylon, work on enzyme has been proceeding, and as this Department was approached for information by the Tea Technologist of the Tea Research Institute, it has been considered advisable to append to this report the work on this subject carried out by the Assistant Scientific Officer. This work was executed during the year 1934–35; it was not published, as it was a scientific work, but it appeared in a thesis for the Ph.D. degree awarded to Dr. Jones. Again the objects in appending this investigation to this Report are to bring the work of the Department completely up to date, and to proffer the information to other Experimental Stations.

Visitors:—In addition to visits from Drs. Wellensiek and Tubbs, the Station also received external visits from Dr. Le Pelley, Entomologist in Kenya, Dr. Tothill, Director of Agriculture, Uganda, and Mr. King, representative of the Dangri Tea Seed Company.

Coffee:—During the year the subject of Quality in Coffee was taken up afresh and the Coffee Product Committee and Mr. James invited Dr. Shaw to give his advice on the subject. It was contemplated asking the Government of Mysore to submit a scheme of work on coffee quality to the Imperial Council of Agricultural Research, requesting the Council to finance the scheme. Dr. Shaw was asked to draw up a scheme which would assist the Director of Agriculture, Mysore, in formulating a final scheme for submission to the Imperial Council. Dr. Shaw's memorandum comprising some 58 pages was forwarded to the Director of Agriculture, Mysore in June 1937; it was a detailed scheme intended not only to assist for Imperial Council purposes, but also in the ultimate execution of the scheme, if sanctioned by the Council.

In this connection visits were made in June to Bangalore to confer with the Director of Agriculture, Mysore, regarding the drafting of the final scheme on broad lines for submission to the Imperial Council. Also at the request of

the Imperial Council, Dr. Shaw served on the ad hoc committees, held in New Delhi in November, to consider the Coffee Quality Scheme and incidentally a scheme for the extension of Cinchona in India.

Dr. Shaw has also been approached by the Imperial Council of Agricultural Research with the request that he undertakes to write the Coffee Section of the Council's Annual Report. With the sanction of the Tea Product Committee this work has been taken in hand, and the Report has been forwarded to the Secretary of the Imperial Council of Agricultural Research.

Rubber:—Owing to the relinquishing of the Rubber Experimental Station at Mundakayam, there existed an accumulation of correspondence files and records. As it was felt that some material of value might be present in them Dr. Shaw was approached by the Chairman of the Rubber Product Committee to undertake the extraction of the scientific material. The files and record books were received in December 1937, and it was immediately appreciated that to deal with them satisfactorily, a survey of the progress of experimental work on the Mundakayam Experimental Station would be required. It was therefore decided to draw up a memorandum which would incorporate any results of value, and coincidentally give a brief history (as far as the short time at Dr. Shaw's disposal would permit) of scientific work on Rubber in South India. A memorandum of some 130 pages was drawn up representing a continuous record of Rubber experimental work, and this has been submitted to the Chairman of the Rubber Product Committee.

A few queries received from Rubber Planters on Pests and Diseases have been answered.

Cardamoms:—During the year a number of enquiries concerning the manuring and diseases of cardamom have been received. Some cardamom soils were analysed in an endeavour to determine characteristics which are conducive to good cardamom development, and from the few results obtained it would appear that cardamoms prefer a soil acidity considerably less than tea, and even coffee. The most interesting aspect of the cardamom enquiries has been the isolation of an eelworm existing in the aerial parts of the cardamom plant, and also a 'wet rot' caused by Rhizoctonia solani. A detailed account of these appear in the Mycologist's report.

It is to be recorded that the approval of the Tea Product Committee has been received for providing the foregoing assistance to Coffee, Rubber and Cardamom Planters.

Acknowledgements:—It is a pleasure to record the help accorded by the Agents and Superintendent of Lockhart Estate to Dr. Jones during his month's visit to the factory to conduct experiments, and to Mr. Murphy, for the facilities he placed at the disposal of the Entomologist for his experiments on Shendurni estate dealing with the control of Helopeltis.

Thanks are also due to the following Planters who, at my request, kindly assisted, by extending hospitality, and giving time in demonstrating South Indian field methods and tea types to the various external visitors to the Experimental Station:—Messrs. Fowke, Jodrell, Hay, Finlayson, Mackay, Greig and Scott-Hart.

Acknowledgements for assistance in the identification of specimens are made to the respective Directors of the Imperial Institute of Entomology and of

Mycology, and to the Staff of the Agricultural College, Coimbatore. The Department has continued to receive the benefit of the loan of technical books through special facilities offered by the Principal of the Agricultural College, Coimbatore.

Conclusion:—As this represents the final report of the present Chief Scientific Officer, he offers no apology for taking this opportunity of expressing his deep sense of appreciation of the many kindnesses and hospitality received from Planters in South India during his thirteen years' association with them.

I also wish to record publicly my thanks to the various members of the Station Staff,—Technical, Clerical and Field,—who have, without exception, supported me loyally by the energetic execution of their duties, throughout the period they have been attached to the Staff.

W. S. Shaw,
Chief Scientific Officer.

SUMMARY OF COMPOST ANALYSES

	No. of Samples	Moisture %	Loss on Ignition %	Nitrogen %	C/N ratio	Phos-phate %	Potash %
			KANNAN DEVANS				
1.	10	51.6	14.2	.43	13.5	.23	.88
2.	2	34.8	19.8	.65	15.2	.27	1.10
3.	1	44.2	17.1	.46	16.5	.43	1.42
4.	1	64.2	12.8	.41	12.8	.14	.97
5.	1	57.1	13.4	.39	16.7	.22	.76
6.	3	49.2	14.5	.45	12.0	.21	.72
7.	1	48.8	13.0	.50	11.2	.49	1.10
8.	6	63.0	14.7	.44	15.4	.18	.75
9.	3	60.8	14.5	.53	12.6	.21	.65
			CENTRAL TRAVANCORE				
1.	8	35.0	16.0	.48	14.5	.21	.58
2.	1	63.7	14.2	.47	14.0	2.15	1.52
3.	4	30.9	12.7	.35	11.6	.19	.71
4.	2	29.7	12.2	.32	12.0	.20	.70
5.	6	46.1	16.6	.46	16.5	.21	.57
6.	9	42.6	14.8	.51	12.2	.31	.86
7.	2	35.4	13.0	.32	16.1	.12	.34
8.	4	37.1	23.5	.85	13.3	.33	.86
9.	3	34.0	20.9	.73	14.1	.29	.90
10.	7	56.3	18.5	.64	13.3	.22	.92
11.	1	24.5	20.8	.71	13.1	.24	.79
12.	6	50.6	16.1	.45	15.6	.21	.63
13.	5	18.1	27.6	1.20	10.1	.41	.83
14.	7	46.1	13.6	.49	11.5	.34	.93
15.	2	17.8	19.5	.46	12.2	.12	.55
16.	6	48.3	16.9	.51	14.5	.43	.68
17.	2	71.9	11.8	.29	17.5	.08	.28
			SOUTH TRAVANCORE				
1.	11	39.1	13.9	.45	12.3	.23	.71
2.	3	51.1	17.4	.60	13.3	.36	.94
3.	2	52.3	16.0	.63	10.9	.21	1.05
4.	3	62.1	16.2	.68	10.6	.27	.82
5.	5	55.8	15.9	.55	12.6	.31	1.10
6.	8	57.9	23.9	1.05	11.9	.20	.69
7.	1	54.5	19.4	.82	11.3	.38	.88

	No. of Samples	Moisture %	Loss on Ignition %	Nitrogen %	C/N Ratio	Phosphate %	Potash %
			WYNAADS, NILGIRI AND MALABAR				
1.	1	63.7	26.6	1.15	10.9	.17	.83
2.	3	57.0	13.3	.45	13.7	.20	.56
3.	2	51.0	25.2	1.09	10.4	.43	1.32
4.	5	51.9	15.0	.53	13.0	.28	.77
5.	4	49.5	19.0	.88	11.7	.26	1.22
6.	1	49.8	15.9	.52	12.0	.30	.81
7.	4	35.8	20.4	.71	11.9	.30	.97
8.	4	50.2	18.0	.71	10.6	.47	1.40
9.	6	49.2	16.6	.57	13.2	.25	.70
10.	11	51.4	15.6	.54	12.7	.25	.77
11.	4	41.3	15.6	.45	14.0	.20	.44
12.	2	45.0	19.5	.66	13.0	.42	.86
13.	1	59.6	14.8	.77	9.4	.37	1.23
14.	5	20.9	17.3	.61	10.7	.54	1.70
			OTHER DISTRICTS				
1.	2	54.0	14.0	.43	14.3	.23	.39
2.	4	52.8	14.0	.45	15.5	.27	.63
3.	1	24.7	15.3	.54	11.5	.26	.47
	No. of	estates	—50.	No. of	samples	—196.	
	Average	47.2	16.4	.57	13.0	.29	.81

PLOT DETAILS

OLD TEA

Month	Green Leaf		Made Tea Per Acre	
	During Month	Total	During Month	Total
April	345		29	
May	794	1139	66	95
June	865	2004	72	167
July	593	2597	49	216
August	893	3490	74	290
September	842	4332	70	360
October	732	5064	61	421
November	843	5907	70	491
December	557	6464	46	537
January	513	6977	43	580
February	468	7445	39	619
March	80 (1 round)	7525	7	626
Average yield over 12 years				437

1926 PLOT YIELDS

Pounds of Made Tea Per Acre.

Month	Sub-Plot No.					
	1	2	3	4	5	6
April	15	7	11	9	10	42
May	145	133	100	111	97	74
June	77	65	67	61	54	81
July	100	94	78	76	61	57
August ..	94	71	62	55	46	66
September ..	187	170	136	126	111	92
October ..	115	80	71	59	59	65
November ..	105	101	101	96	90	88
December ..	101	63	65	62	50	46
January ..	97	73	77	74	50	53
February ..	57	54	74	54	99	60
March ..	9½	9½	9½	9	13	19
Total ..	1102½	920½	851½	792	740	743

During the year this area was divided into 16 sub-plots over which the following four treatments were distributed:—

1. Nil.
2. Compost at rate of 5 tons per acre.
3. Fresh Green Manure (Crotalaria usaramoensis) at rate of 10 tons per acre.
4. Artificials at rate of 350 Pounds per acre (2 : 1 : 1).

A similar series of experiments was commenced on a neighbouring estate.

1927 PLOT

Sub-Plot	Yields in pounds of Green Leaf		
	Half Section A	Half Section B	Total
1	128	150	278
2	95	98	193
3	107	87	194
4	113	78	191
5	178	60	238
6	192	84	276
7	152	98	250
8	203	134	337
9	163	145	308
10	170	141	311
11	149	125	274
12	137	122	259
13	140	121	261
14	125	115	240
15	134	107	241
Total	2186	1665	3851

This is equivalent to a yield of 1013 lbs. of made tea per acre.

The treatment given to the Sub-Plots are as follows:—From June 1937.

1. Clean weeding and no other Cultivation .. 1b, 3a, 7a, 9b, 11a & 15b.
2. Clean weeding with annual deep Cultivation .. 2a, 4b, 7b, 9a, 11b & 14a.
3. Sickle weeding, during monsoon with annual deep Cultivation .. 1a, 2b, 8a, 10b, 13b, 15a.
4. With annual deep Cultivation .. 4a, 5b, 6a, 8b, 12b, 13a.

1928 PLOTS

	15 Betjan	16 Dangri	17 Dhonjan	18 Nellimunda
Section 1 ..	449	484	453	589
,, 2 ..	508	347	453	571
,, 3 ..	418	406	418	491
,, 4 ..	484	453	454	451
,, 5 ..	488	520	539	589
Average ..	469	442	463	538

Pruned early in the month of April 1937 and brought into plucking in October 1937.

PLOTS 19—22

Year	Average Yields		
	19 and 21 High Pruned	20 and 22 Low Pruned	15 to 18 Low Pruned
1930/31 ..	56	..	..
1931/32 ..	183	88	66
1932/33 ..	636	344	395
1933/34 ..	770	706	755
1934/35 ..	402	279	450
1935/36 ..	965	943	1078
1936/37 ..	1449	1396	1310
1937/38 ..	514	521	478

Pruned early in April 1937 and brought into plucking in October 1937.

1928 CONTOUR

Sub-Plot	23	..	296 lbs. made tea per acre.
,,	24	..	131 ,, ,, ,, ,, ,,
,,	25	..	255 ,, ,, ,, ,, ,,
,,	26	..	362 ,, ,, ,, ,, ,,

Resting—August and September but plucked once in each month. 1/4 of the area of Sub-Plot 24 was given a light pruning in January 1937 for the purpose of Branch Canker experiment.

1929 PLOTS

These sub-plots are in the second year from pruning and gave the following equivalent yields:—

Sub-Plot	27	..	1135 lbs. made tea per acre.
,,	28	..	981 ,, ,, ,, ,, ,,
,,	29	..	1015 ,, ,, ,, ,, ,,
,,	30	..	1095 ,, ,, ,, ,, ,,
,,	31	..	1117 ,, ,, ,, ,, ,,

1930 PLOTS

Yield obtained were as follows:—

Sub-Plot	33	..	316 lbs. made tea per acre.
,,	34	..	537 ,, ,, ,, ,, ,,
,,	35	..	634 ,, ,, ,, ,, ,,
,,	36	..	384 ,, ,, ,, ,, ,,

The plots were pruned in the month of October 1937 and the first Tipping took place on the 8th March 1938.

METEOROLOGICAL.

Rainfall during April and May was fairly satisfactory. The South-West monsoon was generally light. It was rather weak, if steady in the beginning and June, though rain fell on 26 days, was characterised by the absence of heavy showers. July provided normal rainfall but August and September were again much below average and a form of drought was experienced. October was remarkable in having experienced 15.95 inches of rain,—a record fall for the month,—of which 5.59 inches fell in 24 hours,—again a Station record for a single day. Rainfall during November was satisfactory and in December the rain usually anticipated about Christmas was also in evidence. January 1938 was absolutely rainless and the few showers received in February were probably too light to be useful. March was again almost rainless.

The year on the whole registered a total rainfall about 6 inches below the average for the previous ten years, the lightness of the South-West monsoon and the absence of rain during August and September being mainly responsible for this shortage. Nevertheless, the monthly distribution was fairly good except as anticipated during the period January—February—March 1938 when a few showers were desirable.

As a result of the distribution of rainfall, the temperatures were comparatively moderate except during March when the maximum temperature rose abnormally on a number of occasions. The minimum temperatures were generally lower than those of last year except in the months of February and March. The minimum temperature of 52°F, probably the lowest on record for the Station, was reached during the year on three consecutive nights in the early part of December. The relative humidity and vapour pressure were slightly in excess, but cloud was generally in defect.

METEOROLOGICAL
(1937-38)

Month.	Rainfall.	No. of days with rain.	Average Afternoon Maximum.	Average Overnight Minimum.	Humidity		Vapour Pressure		Cloud	
					8-30 A.M.	3-0 P.M.	8-30 A.M.	3-0 P.M.	8-30 A.M.	3.0 P.M.
April ..	4.74	9	82.9	65.0	85.9	69.8	22.8	23.0	2.4	4.8
May ..	5.44	15	81.8	64.8	92.0	76.0	24.0	25.0	3.8	4.0
June ..	11.17	26	74.0	64.2	91.5	92.5	22.9	23.6	7.0	5.8
July ..	20.59	31	70.8	63.6	97.8	93.9	22.5	23.1	8.9	8.8
August ..	7.32	17	75.8	62.9	94.3	80.4	22.4	23.2	8.0	5.5
September	5.26	20	76.6	62.5	90.0	81.3	20.9	23.2	2.9	4.4
October ..	15.95	19	75.8	63.2	93.6	81.0	22.3	22.4	4.7	7.0
November	3.65	11	75.0	62.0	87.7	74.7	20.5	20.5	4.6	6.0
December	1.18	3	76.1	58.0	91.6	65.0	18.4	18.1	2.6	3.1
January ..	—	—	78.5	57.3	90.0	47.7	17.6	16.3	0.4	0.3
February	0.72	6	81.7	62.3	92.0	62.2	20.4	16.0	2.6	4.2
March ..	0.17	3	86.0	63.0	83.5	54.3	21.6	20.2	0.9	2.2
Total ..	76.19	160								

REPORT OF THE ENTOMOLOGIST

Advisory:—Enquiries relating to *Helopeltis* were the most prominent during the year, the other pests of tea regarding which enquiries were received being Shot-hole Borer, Termites, Tea Aphis and *Laspeyresia leucostoma.* Reports of Nettlegrubs were absent with one exception, viz., *Parasa lepida* on Aleurites montana.

Cockchafer grubs, *Myllocerus* sp. and scolytid beetles were reported on Grevillea and an unidentified caterpillar borer on Casuarina in addition to other insects of minor importance on diverse plants.

TEA.

Helopeltis:—Enquiries received during the year concerning this pest were far more in number than in previous years. Through the courtesy and assistance of Mr. J. J. Murphy, an opportunity was available for carrying out on Shendurni estate in South Travancore further work in the matter of control. In view of the importance of the problem it has been decided to reproduce here in detail the report embodying the results of various spraying and dusting trials conducted there.

Observations on Helopeltis Control Work undertaken on Shendurni Estate.

The visit to Shendurni estate was made in the latter part of October in connection with the control of Helopeltis. A large scale control campaign was being conducted by the management and it was desired that proper trials be made with the various insecticides that had been obtained for the purpose. A stay of about two months was made on the estate, during which time Fish-oil insecticidal soap, 'Hillo', Lime-sulphur, H. G. Ionised Insecticide and bar soap were the wet sprays tested, while 'Helopelticide', 'Nicosect' and Pyrethrum were the dusts that were given trial.

Fish-oil insecticidal soap:—By far the largest acreage was treated with this material, firstly at a strength of 2%, later increased to 5%. Although these strengths gave effective kills in laboratory trials with captured insects, the result of the experiments under field conditions was in the main negative. Even as many as five applications at intervals of 10 days were not of much assistance in keeping the field free from the pest. Although spraying kills a large number of larvae, a good percentage of adult insects invariably escape unhurt. This was tested by sending podians behind the spraying coolies to search for insects from sprayed bushes; quite a large number of insects, mostly adults, could thus be collected. The search being made within a very few minutes after the spraying of a bush, the possibility of migration from adjoining areas was practically eliminated.

It is needless to enter into all details of spraying here as these have been elaborately discussed in my previous report on spraying experiments conducted on Ambanaad estate with the same material. It may suffice to mention that the present trials have afforded further evidence in confirmation of the conclusions reached during the Ambanaad experiments.

'Hillo':—Preliminary spraying carried out with this material on captured insects gave the following results:—

Dilution	No. of insects employed	Number of insects killed at the end of					Percentage mortality at the end of 6 hours
		2 Hours	4 Hours	6 Hours	18 Hours	24 Hours	
1 in 40 ..	13	..	..	4	..	..	30.8
1 in 30 ..	17	0	0	0	7	15	0
1 in 20 ..	16	..	..	6	..	..	37.5
Control (water sprayed) ..	14	0	0	0	2	3	0

Immediately after spraying, in the first three lots sprayed as above with 'Hillo', all the insects except 2 or 3 were stupefied but when the liquid dried on their bodies, most of them recovered and began to feed. A maximum of 6 hours was considered to be the proper time to observe the mortality due to the application of the insecticide as any deaths occurring thereafter may be due to various other causes. Further, it is also unlikely that any insecticide which has not had any visible effect on the insect up to 6 hours after treatment would exert its action thereafter.

It is seen from the above figures that even at a strength of 1 in 20 'Hillo' is only partially effective, 37.5% mortality being obtained. Under field conditions, a smaller percentage kill would doubtless result. A stronger solution was not tried, as even if successful, the cost of treatment would be prohibitive since a gallon of 'Hillo' costs Rs. 3.

However, a field about five acres in extent, was treated at a dilution of 1 in 30 and although three applications were made, no beneficial results ascribable to this treatment were evident. The cost of treatment at this strength would roughly be Rs. 17/8 per application, for material alone, but in view of its partial utility, it is unlikely that this product would be of much assistance as a means of control.

Lime-Sulphur:—Trials made with this product indicated that at the dilution at which it was being used (i.e., 1 in 30), the material, while being only partially effective against the insect, was strong enough to scorch the tender buds. However, it was observed that an improvement did become manifest during the next few days after spraying, the bushes putting forth fresh shoots. This may perhaps be explained as being partly due to physiological causes and partly to the relief given to the buds and growing leaves from the depredation of young nymphs which are killed by the spray. Some days later, after a round of plucking, the same improvement was not maintained, and the bushes reverted to the same condition as existed before spraying.

Lime-sulphur has long been used on tea estates as a general spray, it being claimed to be effective against fungoid diseases of leaves and shoots at ordinary strengths and as a bark wash for the main stem and branches at greater strengths. It has also been recommended for Red Spider. As an insecticide against Helopeltis, this product was at one time tried in Assam but found to effect no distinct

improvement in the treated area so far as the number of insects was concerned. It was concluded that "applied regularly throughout the season on small areas which never suffer very severely, the amount of damage done can be reduced. Beyond this, there seems to be little hope of spraying ever being of much assistance in the fight against Helopeltis".

The results observed on Shendurni were not far different from the above finding. Its other advantages apart, lime-sulphur is not considered to be of much value as a means of control of Helopeltis.

H. G. Ionised Insecticide:—This proprietary product, claimed to have been prepared with a pure pyrethrin base and ionised oil, was the subject of several laboratory trials and also of a small scale trial in the field. It is readily miscible with water and trials were made at different dilutions.

According to the manufacturers, the average working strength of the solution is 1 in 400, but at this strength it was found to be entirely innocuous to Helopeltis. The following strengths were therefore tried against captured insects and the results are noted below:—

1 in 200:—Immediately after spraying, the insects were not killed. Some died after a few minutes while others remained active and kept feeding for several hours. Observations made the next morning (i.e. 16 hours after spraying) showed that 8 out of 38 insects were still alive and feeding. At the end of 24 hours, 5 insects were found to have survived the spray.

1 in 100:—8 out of 13 insects treated were alive at the end of four hours and 7 till the following morning (i.e. 16 hours after spraying). One of the nymphs that had been sprayed moulted during the night and was feeding normally when observed the next day.

1 in 50:—28 adult insects were treated at the above dilution. Immediately after spraying, they began to move about and seemed apparently unhurt. Within an hour, all except one female showed symptoms of succumbing to the effect of the spray. Some died while others were only making feeble movements. At the end of two hours, all the insects were dead, except the one female insect noted above, which had presumably escaped the spray or was only very lightly hit.

In all the above trials, control insects sprayed with ordinary water were also kept under observation for comparison and there was very little mortality amongst them during the period under observation.

Under field conditions, a small block was sprayed at a dilution of 1 in 50. A search ordered a few hours after treatment resulted in a catch of 14 insects (8 females, 5 males and 1 nymph) in the treated block as against the same number of insects (7 females, 3 males and 4 nymphs) in the control block consisting of almost the same number of bushes as the treated block.

It is thus seen that in laboratory trials 1 in 50 is an effective dilution but under field conditions even this strength is not effective in dealing with all the insects present on the bushes. This result cannot of course be due to the inefficiency of the insecticide to kill the insect but has to be ascribed to the limitation of the act of spraying itself, whatever the nature of the insecticide. However, assuming that 175 gallons of the spray mixture is necessary for treating an acre of tea, $3\frac{1}{2}$ gallons of the product is needed, which at Rs. 32/8 per gallon would cost Rs. 113/12 per acre per application. This is definitely prohibitive. Further, the benefit to be derived appears to be no better than spraying with insecticidal soap which is considerably cheaper.

Bar soap solution:—At 2% strength, bar soap was found to possess better killing property than the same strength of the fish-oil-insecticidal soap. Since it costs roughly Rs. 5 less per cwt. than the latter, it can be more profitably employed wherever spraying can be expected to do good. A block of tea surrounded by a belt sprayed on three sides by insecticidal soap (5%) and one side by lime-sulphur was treated with 2% strength of bar soap solution. The results, as judged by the number of insects caught in the area immediately after spraying, indicated that spraying with bar soap also suffers from the same draw-back as with others tried previously, in that it also leaves a large number of insects unhurt under field conditions.

General Observations on Spraying:—In considering the value of spraying as a means of controlling Helopeltis, the condition of the bushes, the severity of the pest and the time of treatment are important factors to be taken into account. To be able to apply the spray thoroughly, the bushes must be not more than 6-7 months from pruning. Full-grown bushes with a dense crown of leaves render the work of spraying difficult and the efficiency of the operation is further impaired by the fact that the bushes are invariably disturbed before spraying by the passage of the cooly from bush to bush.

The severity of the pest is the next point to bear in mind. In areas which never suffer very severely, spraying may be attended with good results but in places where the pest attains serious proportions, it appears most unlikely that this operation will result in any considerable dimunition of the pest.

The third factor, viz., the time chosen for undertaking control operations, so affects their value that its importance cannot be over-looked. In the early part of the year when the number of insects is small, spraying, if undertaken, may be expected to effect a reduction in the number of insects that would avail themselves of subsequent more favourable conditions. Consequently, the incidence of severe attack may be delayed. On the other hand, in the middle of the season either when the infestation is advancing or, even if declining, when the insects are still present in large numbers, any attempt with spraying is most unlikely to succeed. A large number of insects may be killed by the spray but as there would still be many insects left unhurt, especially the adults, further infestations may proceed practically unaffected by the previous applications of the spray.

The other aspects of spraying, especially the limitations that detract the value of spraying, have been discussed in detail in my previous Report on Spraying Experiments undertaken on Ambanaad estate (April-July 1936).

The present trials using different insecticides, often at frequent intervals, though extensively carried out, have unfortunately failed to give a measure of success commensurate with the expense involved. It must be stated that this lack of success has not been due to any quality of resistance in the insect but to failure, purely on mechanical grounds, to cover the plants effectively and quickly. The bushy habit of tea makes it virtually impossible to hit the undersides of all leaves with the liquid. This would leave a number of larvae unhurt in addition to a much larger number of adult insects which, being provided with wings, would obviously escape on the approach of the spraying cooly.

It is my considered opinion that no very complete control of Helopeltis, under field conditions, is likely to be achieved by spraying until a considerable advance is made in the technique of the application of insecticides. In view of the serious results attending continued infestation, strenuous efforts should be made to solve this very urgent problem. In addition to devising suitable technique

for effectively dealing with insects present on the bush, the problem of rapidly covering large areas should receive further investigation with a view to reduce the effects of migration of the insect from untreated areas.

Dusting Experiments.

"**Helopelticide**":—This material was offered for trial by Messrs. Shaw Wallace & Co.

Area chosen for experiment:—A small block, roughly half an acre in extent and situated near the jungle border, was demarcated for dusting at the farthest end of the Old Bungalow field,—an isolated field of about 14 acres. This block consisted of 456 bushes in plucking and 818 supplies.

The field at the time was not uniformly infested. Most of the bushes showed serious infestation, but some, although in the vicinity of badly attacked bushes, were comparatively free and flushing normally. Such bushes could often be seen in sample patches. In the vicinity of jungle border, both badly attacked and comparatively free bushes could be seen side by side and the theory that jungle border confers immunity to the bushes in its neighbourhood was entirely untenable so far as this field was concerned.

24 bushes showing severe attack in the block chosen for dusting were marked with sticks prior to dusting with a view to observe how far they would respond eventually to the treatment.

Treatment:—The first application was made on the 25th October 1937 with a "Peerless" Rotary Duster. The two-nozzle combination, though capable of quick work, was not suitable for dusting tea bushes as a large amount of dust was found to be aimlessly drifted away. The single nozzle too, in view of the angle at which it was projecting the dust, was rather difficult for the operator to manipulate for dusting the bushes thoroughly from all sides. A nozzle curving downwards was therefore got made locally by a blacksmith and dusting was done by fitting this to the end of the machine. This arrangement worked satisfactorily.

It was drizzling early in the morning when the application commenced but very good weather prevailed throughout the period of dusting. 0.31 inch of rain fell on the night following the treatment.

The second application was made on the 4th November, 10 days after the first round. The weather was bad over-night with rain and wind which persisted till the early hours of the morning. After 9-30 a.m. the weather cleared and work was undertaken at 10-30 a.m. in bright sunny weather. Except for occasional strong breeze, the actual dusting must be said to have been carried out in favourable weather. Soon after the work concluded, there was, however, a fairly heavy downpour of rain (1.18 inches) which lasted for a brief duration of 40 minutes. Subsequently, the evening was cool and calm without any rain.

The third application was taken up on the 13th November—9 days after the second. Two dust guns were operated and the area was covered in about 3½ hours. The weather was bright and sunny with occasional slight breezes.

During this round, it was not really necessary to dust all the bushes as most of them were entirely free from fresh bites, but for the sake of thoroughness, all the bushes were treated.

On the whole, it may be said that all the three applications were made in favourable weather.

From the above experiment the following data relative to the economics of dusting have been collected:—

Capacity of 'Peerless' dust gun	8 lbs.
Number of bushes dusted per fill, average	150.
Time taken to discharge one fill	1 hour 45 mins.
No. of bushes a cooly can dust in a day	750—800.
Quantity of dust per acre	128 lbs.

Observations:—Preliminary trials with captured insects indicated that the dust was 100% toxic to the insect. Even in the field, two insects 'in copula' were observed to receive a thin coating of the dust which was sufficient to kill them. With a view to determine the efficiency of dusting under field conditions, two podians were sent to collect insects in the dusted area a day after the first application, under personal supervision, when 37 adult insects and 11 nymphs of all stages were captured. Obviously, some insects escape the dust, though probably not in such large numbers as with a liquid spray. Fresh bites were also noticed on some of the bushes.

Several insects that were collected had the dust still adhering to their bodies but they seemed to be none the worse for it. It would thus appear that the effect of the dust is only temporary and that such of the insects as are only very lightly hit and do not succumb within a short period will recover eventually from the effect of the dust.

At the time of the second dusting, fresh bites were comparatively few and the search made while dusting was proceeding (podians following behind the dusting cooly) did not reveal a single insect on the dusted bushes even when the search was extended to 5 or 6 hours.

The following morning, a search was again made in the dusted area as also in an approximately equal area in its neighbourhood. The latter at the time had been sprayed twice with insecticidal soap. In four hours, 10 insects were caught in the dusted area whereas in the adjoining sprayed area 36 insects were obtained.

Observations a week after the second dusting indicated that the dusted area was, on the whole side, looking remarkably well. Stray bites by adults and young larvae hatching subsequent to dusting were, however, noticeable, but these fresh attacks were very slight. The badly infested bushes marked with sticks had thrown off the pest almost completely and were starting to flush normally.

During the third application, it was noticed that the response made by the majority of the bushes was so good that it was hardly necessary, as previously mentioned, to dust all the bushes.

After the third dusting, the area was looking very good indeed. The majority of the 24 marked bushes had improved remarkably and it was hard to believe that they were really the very badly attacked bushes before dusting. There were still fresh bites on some bushes but compared with the neighbouring area which had by then received three applications of insecticidal soap, they were very few.

Three weeks after the third application, the area, in spite of cropping satisfactorily, was observed to have fresh attacks more generally distributed than a week previously. Some bushes were showing definite symptoms of increased infestation, while others were still either free or having only very slight attack.

A week later, the pest was observed to have gained ground. Being nearly a month since last treated, it was to be expected that such of the insects as had survived the dust had multiplied subsequently unhampered, assisted also in some measure by insects migrating from the adjoining area. Although crop records are not exclusively a reliable guide in assessing the value of any treatment for the control of Helopeltis, still they were kept as auxillary data and are reproduced here.

Crop harvested after dusting.

4th November	..	14 lbs. green leaf.
11th „	..	21 „ „ „
18th „	..	28 „ „ „
25th „	..	28 „ „ „
3rd December	..	34 „ „ „
10th „	..	20 „ „ „

Conclusions:—"Helopelticide" is definitely toxic to the insect, both adult and larva, and provided a bush is thoroughly dusted, theoretically two applications should be sufficient for obtaining a good measure of control.

Improper storage and exposure to light and air would adversely affect the insecticidal efficiency of the dust. Dusting was done thoroughly, spending as much as 128 lbs. per acre, but even then complete control was not obtained, as a few insects were always found to survive each application. This may not be so much attributed to the lack of efficiency of the dust as much as the difficulty of applying it efficiently to full-grown tea bushes. Technique of applying the dust also needs to be improved upon. It is possible that with an improved device for applying the dust, a much smaller quantity than 128 lbs. may be sufficient. The cost of the product is not known, but even should it be cheap compared with other insecticidal dusts, dusting, as at present carried out, would be an expensive operation.

"Nicosect":—This again is a proprietary dust, probably having a nicotine base, and was recommended by the manufacturers as being best applied with sulphur and lime as diluents in the following proportions:—

Sulphur	.. ..	8 lbs.
Hydrated lime	.. ..	10 „
"Nicosect"	.. ..	1 „

Area chosen:—was a small plot in the Upper Bungalow field near the edge of the jungle. A block consisting of 109 bushes disposed in 7 rows was marked out for experimental dusting, while a similar number of bushes was marked as control in two parts, one on either side of the dusting area. The areas were bounded on one side by jungle and by tea on all other sides. At the start of the experiment, the areas were severely attacked by the pest and nearly 80% of the bushes were more or less in a 'shut up' condition. They were of a poor jat, probably china hybrid. The experimental and control plots were almost uniform both in the matter of infestation and growth.

Treatment:—The areas were weeded just prior to dusting. 12 flags were put both in the experimental and control areas, each flag between two bushes, so that observations could be made on 24 bushes in each area.

The first dusting was made on the 15th November, in favourable weather, with a 'Peerless' rotary dust gun. The insecticide was employed in combination with locally ground sulphur and lime in the ratio of 1 : 8 : 10 by weight.

The second application was made on the 24th November, 9 days after the first round. There was a fair breeze throughout the period of dusting and as a result, the control plot towards one side must have had a small share of the dust.

Observations:—Preliminary trials indicated that this material is toxic to both young and adult insects, though, however, death was not so rapid as with "Helopelticide". Immediately after dusting, none were killed and some fed for a time. At the end of three hours, however, 90% mortality was noted.

24 hours after dusting in the field, no fresh attacks nor insects could be discovered in the experimental plot. Fresh attacks and also a few insects were found in the control area.

Five days after dusting, the first of the weekly observations was made on the bushes marked by flags and it appeared, on the whole, that except for new bites made by young larvae hatching from eggs subsequent to dusting, the experimental area was practically free. A search made for two hours revealed 2 adult insects in the dusted area as against 14 in the control. Tiny larvae hatching subsequent to dusting were not taken into account although they were commonly met with in both areas.

Just before the second application, it was observed that new shoots were coming up well in the treated area in spite of a fair amount of fresh attack by young larvae on some bushes. Compared to the control plot where fresh attacks were very common, the treated area appeared to have made very good recovery.

The tiny larvae which were noticed on previous days were not to be seen after the second dusting. Although it could not be said that there were no adult insects, their number, however, was almost negligible.

A day prior to the third application of the dust, the area was again examined. The treated area was looking remarkably well, though slight attacks were still noticed here and there, more especially in rows adjoining the control blocks.

The weekly observations made on individual bushes indicated, generally, that the bushes threw off the attack and improved considerably as a result of dusting. The crop records kept during the period of the experiment offer further evidence to the favourable results ascribed to dusting. They are as follows:—

	Treated.	*Control.*
24th November ..	2 lbs.	1 lb.
1st December ..	4 lbs. 10½ oz.	1 lb. 13 oz.
8th December ..	4 lbs. 14½ oz.	2 lbs. 4 oz.

It is seen that the dusted area, a fortnight after the commencement of dusting, yielded roughly 2½ times more leaf than the control. There was an increase over the previous round, from 2 lbs. to 4 lbs. 10½ oz., whereas the control showed an increase of 13 oz. This increase in the control plot may perhaps be attributed to climatic conditions.

The yield on 8th December showed an increase in both areas, the treated plot still maintaining more than double the crop obtained from the control.

Conclusions:—The results of 'Nicosect' dusting appear, on the whole, satisfactory; but it must be stated that, with the material available, only a small scale trial could be undertaken. The cost of the product is not known and the economics of dusting with 'Nicosect' is therefore omitted from discussion here.

Pyrethrum:—This material of Yugo-slavian origin was the subject of a fairly extensive trial in the field, in addition to several small scale and laboratory trials varying the nature and quantity of the diluents.

Site:—The area employed for dusting was a block of about 4 acres, the tea being more than two years from pruning. Although it was realised that it was not a quite suitable area, no other choice of a more suitable area was available since every other field had already been treated with one insecticide or the other by the time the Pyrethrum arrived on the estate. The area was bounded by jungle on three sides, (west, south and a part of east) and by tea on the north and part of east. Being fairly steep, the lower portion of the area situated on the southern aspect towards the jungle was more or less protected from wind. The bushes there were in a much worse condition than those towards the top. The area, on the whole, may be said to have been severely attacked, with many bushes (about 30%) in a practically 'shut up' condition. Some, however, though badly attacked, were showing signs of putting out fresh shoots.

Treatment:—A mixture of pyrethrum and lime in the proportion of 1 : 2 by weight was employed for dusting in the field, this proportion having been adopted after making preliminary trials which were successful in killing the insects.

The first application started on the 17th November with two dust guns and the area was completed in 5 days. The second round commenced on the 27th November, 10 days after the commencement of the first, and concluded on the 2nd December. The third application was begun after an interval of 10 days but was completed within three days as 3 dust guns were working during this round.

The weather conditions were generally dry throughout the period except for a slight drizzle on one day and 0.39″ rain on another. The latter occurred, however, during the interval between the first and the second round and the actual dusting was therefore unaffected. A fairly strong breeze was occasionally experienced on certain days causing the dust to drift, but the difficulty on this score was not very great. The use of a trailer was at first contemplated but in view of its doubtful utility, added to the slowness of work that its employment would involve, the idea was abandoned. The relative humidity during the afternoons was perhaps low but, whether this has any adverse effect on the efficiency of the dust could not be investigated.

Observations:—Pyrethrum has a high toxic value against Helopeltis. From numerous trials both in the field and with caged insects it was clear that it is capable of killing both the adults and larvae. It could also stand admixture with double its weight of a carrier, as may be seen from the accompanying table of dusting trials conducted in cages.

Five days after the first round, the dusted area was looking better than at the beginning of dusting, with a tendency to throw fresh shoots free from attack. It was, however, found that dusting had not completely eliminated the insects as a fair number of them were still noticeable on many bushes, apart from the bites of young nymphs hatching from eggs subsequent to dusting.

A couple of days prior to the second application, the area did not appear as much improved as was anticipated. Fresh growth had not been quick in coming up, though the buds on many bushes seemed to have had relief from the attacks of nymphs and were developing free from bites.

After the second application, improvement was more manifest, but insects could still be found, though, however, in much smaller numbers. It was therefore found necessary to treat the area to a third round of dusting.

The third application appeared to have still further reduced the number of insects and the growth of free buds was more marked. Even after this round, however, complete elimination of the insect was not to be obtained.

The reasons for this lack of complete success in ridding the field of every insect are not far to seek. In the first place, the bushes being full-grown, it is impossible to dust them without previously disturbing them, thereby giving a chance for the insect to fly ahead and escape. Secondly, with full-grown bushes, anything like thorough coverage is impossible to attain in practice without spending a very large amount of the dust. (Even now, the consumption per acre is definitely on the high side). The insects that drop down to the ground before they are hit are apt to escape, especially if the area be weedy. Thirdly, it is possible that, due to wind often getting strong, the dust may be wafted away before it has had time to permeate the bushes. Lastly, even if single bushes here and there, or parts of them, are overlooked while dusting, the insects present on them would be a source of fresh infection to dusted bushes in addition to those migrating from untreated areas.

In addition to the above trial, several small scale trials were also made, using different combinations of lime and sulphur. Pyrethrum, lime and sulphur in the ratio of 1 : 1 : 1 appeared to give slightly better results than either Pyrethrum and lime (1 : 2) or Pyrethrum and sulphur (1 : 2). It is probable that, as in the case of certain other sucking insects, sulphur has a residual effect adverse to the young stages of Helopeltis and its employment in combination with Pyrethrum would therefore appear useful. However, it is only by long time experiments and observations in the field that we may finally arrive at any definite conclusions.

Finely powdered wood-ash may also be employed as a carrier in place of lime without affecting the toxic value of Pyrethrum.

Conclusions:—The conclusions arrived at in respect of 'Helopelticide' are almost equally applicable to Pyrethrum. It is important that lime or sulphur or whatever carrier is employed must be mixed with the insecticide just before use and not kept readymixed for an indefinite period, as mixtures kept for several days were found to have lost part of their toxic value. The dilutent must be in as finely powdered condition as possible, as then only a thorough mixture could be made with the insecticide capable of killing the insect even if a very small quantity of the dust should stick to its body. Further, fineness imparts lightness to the dust and for a given weight of the mixture a larger number of bushes may be dusted than when the powder is coarse.

The quantity of the carrier to be employed, though determined after biological tests, was arrived at only empirically without any knowledge of the pyrethrin (the active principle) content of the pyrethrum employed. If the Pyrethrum has been accurately assayed, the information in respect of the percentage of pyrethrin contained therein should be useful in deciding with greater accuracy the quantity of the carrier to be added.

The price of pyrethrum being nearly Rs. 1/– per lb., the cost per acre, per application, would roughly be Rs. 40/– for material alone. Further, as applications have to be repeated more often than once, the treatment would be very expensive.

General Observations of Dusting:—Dusting, on the whole, has given decidedly better results than wet spraying. The treatment, however, is more expensive, but so long as it is known that it is of definite assistance, the question would then develop into one as to how to reduce the cost. The factors already discussed under spraying, such as the condition of the pest, the age of tea from pruning and the time chosen for treatment are also factors which affect the efficiency of dusting. Proper attention to these factors should lead to greater efficiency and reduction in costs. More important than all these is the need for improvement in the technique for efficient and rapid application of the dust. Knowledge in this regard is a great desideratum.

Summary:—During the visit to Shendurni opportunity was provided for undertaking trials with various insecticides against Helopeltis.

Fish-oil insecticidal soap, "Hillo", Lime-sulphur, H.G. Ionised Insecticide and bar soap were the wet sprays tested. Although the materials were toxic to the insect, the kill obtained under field conditions was poor in every case. Even as many as five applications, at intervals, were not found to be of much assistance. However great the toxic value of the insecticide may be, in view of the various limitations, wet spraying is considered unlikely to be successful in areas which suffer severely from the pest.

Dusting trials were made with "Helopelticide", "Nico-sect" and Pyrethrum. All of these were toxic to the insect and the results under field conditions were in every case much better than wet spraying, thus indicating the superior value of dusting as a means of Helopeltis control.

The severity of the pest, the condition of the bushes, their age from pruning, and the time chosen for treatment are considered to affect the efficiency of both spraying and dusting.

Dusting, as at present carried out, is expensive and considerable economy must therefore be effected before its adoption could be practicable. The need for improvement in technique for efficient and rapid application of the dust is indicated.

In addition to the above work, some observations were also made on the parasites of *Helopeltis*. The Mermethid worm and the wasp grub reported previously as occurring in Central Travancore were also to be found in South Travancore. The latter, however, was met with more commonly than in Central Travancore and daily catches made during November revealed that as much as 10 to 15% of the fourth and fifth instar nymphs of *Helopeltis* were infected. The cocoon stage appears to be a critical one in the development of the wasp parasite and attempts made towards obtaining the adult wasp have not so far met with success.

In view of the increased number of enquiries received during the year concerning *Helopeltis*, it was suggested by the Chief Scientific Officer that his book on this subject published in 1928 and now out of print might be revised and reprinted. Some time was spent in the preparation of supplemental data for bringing the subject matter up-to-date.

Laspeyresia leucostoma (Leaf Roller):—An outbreak of this pest was reported from an estate in Nilgiri–Wynaad where the insect appeared to have

attacked all the first flush coming in after tipping. The depredation had extended throughout a 17 acre field and was reported to have taken place for the second time in the same field, the first one having occurred two years previously, though in a milder form. Systematic hand-picking was advocated and the pest was probably brought under control by adopting this measure.

In the month of September, the pest became conspicuous on the Station fields when an opportunity was available for a further investigation into the life-history and bionomics of this pest. The eggs which have up to the present eluded observation were discovered for the first time in the field. They are laid, not on any of the leaves of the flush as was believed by previous workers, but on older leaves further down the branch. This is a discovery very important from the aspect of control, as in normal plucking, no eggs of *Laspeyresia* are removed from the bush in contrast with *Gracilaria theivora,* another leaf-roller, where plucking of two leaves and a bud eliminates very nearly 75% of the eggs deposited on the plant. Further points adding to our knowledge of the life history were also investigated and a short resume of the studies so far made is given below:—

The egg which measures less than 1 m.m. along the long axis is almost round or slightly oval and in view of its light yellow colour, is very difficult to observe on the leaf. After the caterpillar has emerged the empty egg shell is more easily seen as an iridescent white spot. The egg is laid singly, though in one instance two eggs were seen overlapping each other. These however were obviously not laid at the same time as the larvae hatched out on different days, nor could it be said definitely that both were laid by the same moth. Only a single egg is generally laid on each leaf, though the occurrence of two on the same leaf is by no means uncommon. Very occasionally, 3 eggs were found laid on a single leaf, two on the upper surface and one on the lower.

The leaves generally chosen for egg-laying are the old, hard and leathery ones, though occasionally fish leaves may provide sites for oviposition. The eggs may be laid on both sides of the leaf but the upper surface is generally preferred. Even here, the basal part of the leaf, i.e., the petiolar end is preferred, presumably to facilitate the emerging caterpillar to climb readily up the shoot to reach the flush.

The newly hatched caterpillar is very active and soon proceeds to the unopened bud. The characteristic hole is bored therein and the caterpillar lodges within the marginal roll. The same thing probably happens in the field also, where in freshly attacked shoots such a hole is almost invariably present.

During the first 4 or 5 days very little feeding is done, but within the next two or three days the characteristic bindings of silk could be seen. The opening of the bud into a leaf and the bindings of the caterpillar seem to proceed side by side. The bindings are made from the tip downwards towards the base of the leaf.

The descriptions of the full-grown caterpillar and the pupa, the mode of pupation, the emergence of the moth, etc., have already been dealt with in the Annual Report for 1932–33, p. 41.

The moths are active on the wing during the evenings, but it is not unusual to come across them at any time of the day. It is difficult to distinguish the sexes with the naked eye as there is no outwardly visible indication of sex. They may, however, be distinguished by an examination under a lens of the last abdominal segment which ends in a feather in the male and in an orange-coloured concavity in the female.

The ratio of sexes was found to be almost equal. Copulation and oviposition habits of the moth still remain obscure. Newly emerged moths were kept under observation and in spite of both sexes being represented in the number kept in captivity, they refused to mate. Several attempts were made and in no case was copulation observed to take place. Consequently none of the moths (over 20 pairs) laid eggs under artificial breeding conditions.

On the basis of present knowledge, control measures can only be directed against the caterpillar and pupal stages. As has already been pointed out, the inconspicuous nature of the eggs, added to the fact that they are laid singly on mature leaves low down the branches, precludes the possibility of eliminating them during normal plucking or by hand-picking. The moths also are difficult to obtain and until we know more about their habits, seasonal abundance and other host plants, no satisfactory method of trapping them can be devised. Trials made in Java have indicated that light traps fail to attract the moths. A systematic hand-picking of infested shoots with a view to destroying the caterpillars appears therefore the only possible method for the present. Owing to the preference shown by the pest to tipping tea, an alteration in the existing cultural practices, such as pruning, with a view to prevent the coincidence of tipping stage with the period of maximum prevalence of the pest, may bring about reduction in damage. But definite recommendations in this regard can only be based on the knowledge of the seasonal history of the pest and this aspect of the investigation is receiving attention.

In addition to the two parasites previously reported viz., *Elasmis homonae,* Ferr. and *Asympiesella indica,* Gir., a few more have been obtained and are awaiting identification.

Gracilaria theivora:—The tea leaf-roller frequently occurring in conjunction with *Laspeyresia* was the subject of further study during the year under report.

The eggs, which are small, oval and transparent, are laid singly on the leaves of the flush, the first and second leaves being usually preferred. On examining hundreds of eggs laid in the field, it was found that the first leaf is the more preferred. They may also be laid occasionally on the III and IV leaves, rarely on the V, but not on the older and harder leaves lower down in the bush, as the emerging caterpillars cannot mine through the tougher leaves. During severe infestation, deposition of eggs may also take place on the bud and the tender stalk between the leaves. The lower surface of the leaves is invariably chosen and eggs are laid with equal facility on either side of the midrib, being more concentrated in the neighbourhood of the midrib and becoming more dispersed towards the edges.

The egg of *Gracilaria* can be easily distinguished from that of *Laspeyresia* by its position (on the tender leaves of the flush), smaller size and oval shape. Though normally a single egg is laid on a leaf, it is not uncommon to find 3 or 4 eggs on a single leaf under field conditions, during a severe infestation.

The caterpillar on hatching does not come to the outside but bores through the bottom of the egg and enters the leaf tissue between the upper and lower epidermis where its mining activities begin. The tortuous courses of a silvery colour often met with on the leaves are the result of the work of this miner.

After a few days spent in the mining stage, the caterpillar which was hitherto flat, in adaptation to its mining habit, moults and assumes a cylindrical body. It then bores its way out through the epidermal layer of the leaf and enters upon a

free-living existence, as a leaf-roller. It then gnaws a hole in the midrib of the same leaf as it was mining or, more generally, of a fresh leaf, about the middle of its length, presumably with a view to facilitate the bending of that part of leaf beyond the hole for its subsequent rolling operation. It lives and feeds inside the roll thus made and this part of the leaf may show slight withering as a result of arrest of sap flow caused by the hole.

When full-grown, the caterpillar generally chooses a spot between two veins on the lower surface of the leaf and a bridge is first spun across them by strands of silk. In the hollow thus formed, the roof of the cocoon is first constructed, the caterpillar working from an upside down position. The faecal matter extruded during the process of construction is deliberately pushed on to the roof in the median line probably to add extra strength to the construction. The hairs present on the area of the leaf enclosed for pupation are also sheared and utilised for reinforcing the roof. The two ends are next closed and the basal part which is now bereft of all hairs and smooth is also lined. The process of cocoon formation is more or less completed in about two hours but the caterpillar can be seen lining the interior for another two or three hours before actually pupating.

The pupal period lasts for 12–13 days. The emerging moth is slender in build, reddish brown and possesses a bright yellow patch on the middle of each forewing. The antennae are long and slender and are kept in rapid alternate movements.

The moths appear to be nocturnal in habit. Unlike *Laspeyresia* they laid eggs in captivity, though, again, copulation could not be observed even here. Egg-laying takes place for nearly a week after the emergence of the female moth.

Gracilaria, though occurring at the same season as *Laspeyresia,* its generations overlap to a less degree than in the latter. The period over which the maximum number of eggs is laid is more or less defined and this fact should serve as a useful clue in the adoption of control measures. Investigations into the seasonal history of the pest are therefore being continued.

From the aspect of control, this leaf-roller occupies a different position from that held by *Laspeyresia.* Unlike the latter, eggs of *Gracilaria* are eliminated in the normal course of plucking and from counts made of eggs laid on different leaves in the field, it has been computed that an average of 70 to 75% of the eggs is removed if a bud and two leaves are plucked.

The caterpillar damage being confined to single leaves is not so great as that of *Laspeyresia* and the removal of attacked leaves during rounds of plucking should act as a further useful measure of control. In this case, there can be no objection against the plucking away of rolled leaves, as the growing shoot is scarcely affected.

Further, parasites often play an important part in the control of this pest. *Asympiesella indica,* Gir., *Dioctes* sp. and *Tetrastichus* sp., are among the parasites so far noticed.

Shot-hole Borer:—Three more estates in the Vandiperiyar District reported this pest during the year. No further information was available regarding the status of the pest in that district.

Through the courtesy of the Entomologist, Tea Research Institute, Ceylon, a collection of stems bored by shot-hole borer was obtained from Ceylon with a view to making comparisons with the type of damage caused by the local species.

The various types of gallery formation and their relation to the thickness of the branches were intended to be compared but much progress could not be made owing to the pressure of other work.

Nettlegrubs:—No serious outbreak of this pest was reported during the year on tea, but report of an attack by *Parasa lepida* on tung oil tree (*Aleurites montana*) came from an estate in Peermade during the monsoon.

Termites:—The situation regarding this pest of the Anamallais remains the same as before and no opportunity was available during the year for undertaking any control measures. Before considering other methods, the Paris Green treatment evolved in Ceylon was suggested in last year's report for trial in South India also, but nothing is known whether any attempts were made in the affected district towards control by this means. H. G. Ionised Insecticide, a proprietary product having in its composition a pyrethrin base and an ionised oil body was reported as having been tried by a group of estates in the Anamallais and found to be sufficiently toxic to the insects at a dilution 1 in 100. It is an expensive product, as judged by the results obtained with *Helopeltis,* but what quantities were required for treatment of termites is not known, nor is any further information available regarding the economics of the treatment.

Coptotermes sp. was reported from an estate in the Anamallais during the year under report and it was said to be fairly common there. The specific identities of the termites attacking living tea bushes in South India have unfortunately still remained undetermined for want of adequate specimens and requests for more material have not met with response.

Tea Aphis:—The periodic attacks which tea suffers from this pest, especially when coming in from pruning, prompted a study into the bionomics of this pest. It is significant that in the case of pruned tea, the bushes that are slow to recover from the effect of pruning, are the worst to suffer from Tea Aphis. It is found that climatic factors, such as rainfall and the presence of parasites and predators exert a profound influence on the growth and dispersal of these insects. Work in these directions was taken on hand but the data, being still incomplete, will be enlarged upon at a later date.

Looper caterpillar (*Biston suppressaria?*). This pest was the subject of an enquiry from the Anamallais where a large number of caterpillars were reported as having completely denuded two or three tea bushes, with evidences of spreading to others. The Geometrids (the family name applied to all loopers) of which there are at least three or four species occurring on tea in South India are generally of sporadic occurrence, but during the years when they are found in large numbers considerable damage may be done. Early detection of the pest and prompt measures of control will often keep them in check. But when they once get out of hand control becomes almost impracticable and the crop may suffer greatly not only during the year of visitation but also, though to a less extent, for one or two subsequent years as a result of recrudescence of the pest. Control measures as are outlined on pp. 39–41 of the Annual Report for 1935–36 may be adopted in dealing with this class of pests.

Scale Insect:—Coccids tentatively identified as *Lecanium formicarii,* Green, were reported as attacking tea in Nilgiri–Wynaad. Unlike the Brown Bug (*Lecanium hemisphaericum*) they are sheltered by the nest of an ant. The report is of interest as it is the first time that this insect has come to the notice of this Department as occurring on tea in South India.

GREVILLEA

Cockchafer grubs were the cause of considerable damage to a large Grevillea nursery in Wynaad. The grubs living in the soil at a depth of 3 or 4 inches below the surface were reported as attacking the roots and chewing them off entirely. The control of this type of pest is often a difficult matter but the use of soil disinfectants such as calcium cyanide, white arsenic or 'Vaporite', a proprietary product, was, however, recommended for preliminary trials.

Scolytid beetles were again reported this year on Grevilleas in the Anamallais. In the material received for examination there were represented at least seven different species of beetles, viz., *Xyleborus semigranosus,* Bldf., *X. andrewesi,* Bldf., *Eccoptopterus sexspinosus,* Mots., *Platypus latifinis* Wlk., and three other unidentified species. Though these insects are generally borers in moist wood, they are considered unlikely to make headway against the flow of gum which is produced in a normally healthy tree. The infestation was considered to be due to a prediseased condition of trees as a result of other causes. Remedial measures against the borers were therefore considered unnecessary.

Myllocerus sp. was once reported on Grevilleas in the Nilgiri–Wynaad but the nature of its damage to the trees was obscure.

ALBIZZIA.

Terias sp. was the only pest of Albizzia reported during the year, the report coming from Central Travancore. It is a pest of sporadic occurrence common throughout South India and may be controlled in the manner described in the Annual Report for 1934–35, p. 24.

The tiny, shining black moth whose yellowish caterpillar was mentioned as feeding on Albizzia in the Annual Report for 1935–36, p. 43 was identified as *Ascalenia liparophanes,* Meyr. (Cosmopterygidae).

Miscellaneous:—An unidentified caterpillar borer was the cause of damage to Casuarina. As tested with a similar borer of Red Gum, injection of a mixture of equal parts of creosote and chloroform into the tunnel should prove useful in dealing with this pest.

Several proprietary insecticides were received for trial and a few enquiries were made of others with a view to their possible adoption against any of the tea pests.

REPORT OF THE MYCOLOGIST

Advisory:—During the year under review one hundred and twenty specimens were received for examination and report. A large number of these related to leaf diseases of Tea and Grevillea. Enquiries regarding stem and root diseases were comparatively less frequent.

TEA.

Leaf Diseases:—Climatic conditions seem particularly to have favoured the leaf disease organisms in general. In addition to Black Rot (*Corticium invisum*) and the Cercosporella leaf disease, an apparently new disease, Black Blight (*Asterina camelliae*) was recorded from a large number of estates.

Cercosporella Leaf Disease:—This disease, first reported during the year 1935, has come into greater prominence during the last two seasons. In the year under report the disease occurred on many estates in the Nilgiris, Wynaads, the Anamallais and the High Range. It is however not known whether estates in the South and Central Travancore districts have also experienced this disease.

The attacks of the fungus *Cercosporella theae* results in appreciable defoliation of bushes and has therefore been a cause of concern to Managers of estates. Although the degree of infection appeared to be greater when tea was growing under *Albizzia* or *Acacia* it could by no means be said that tea shaded or unshaded, was immune. As an extreme case it was observed that tea situated under a Grevillea wind belt had been completely defoliated, while the same degree of leaf fall was not apparent on the same estate under *Albizzia moluccana.* This difference might be the result of the location of that part of the field, or on account of the age of the tea from pruning. Evidence was also available pointing to the previous condition of the bushes having largely influenced the attack; thus weak bushes seemed to have suffered to a much greater extent than their healthy neighbours.

With the limited observations made no correlation between jat of tea and susceptibility to the disease could be made.

An attempt was made to connect the direction of wind and rainfall with the prevalence of the disease. No conclusions however could be arrived at, as there was an almost uniform distribution of the disease in patches spread over areas which were protected, and unprotected, from the direct influence of these two factors.

Control measures such as spraying, although not practicable on an extensive scale, can do considerable good in checking the spread of the disease in the earlier stages, but, for such an undertaking to be effective, the time of application should be appropriate. It may be that a pre-monsoon application, carried out just before the rains, will assist in protecting the foliage from infection.

The fungus was recorded during the year on the following hosts:—*Acacia decurrens, Albizzia moluccana, Poinciana regia* (Gold mohur tree) and *Aleurites* spp. (Tung oil).

Isolations from none of these hosts has given the fertile perithecial fructifications.

Black Rot (*Corticium invisum*):—Only a few enquiries were received regarding this disease, and reference has been made here because of its association with

the *Cercosporella* leaf disease. On practically all leaves received from the Anamallais reported as being attacked by Black Rot, innumerable spores of *Cercosporella theae* were obtained. Because of the capacity of both the organisms to cause serious damage it was not possible to determine which was the primary agent. It is however likely that, owing to the capacity of *Cercosporella* to produce easily disseminable spores in prolific numbers, the presence of these spores is secondary.

Black Blight (*Asterina camelliae,* Syd. & Butl):—Tea leaves often show black discolorations on their upper surface, and this discoloration is the result of an attack from a fungus *Asterina camelliae.* Such discolorations are also caused by other fungi that follow attacks of scale insects and are termed sooty moulds (Vide Ann. Rept. 1935/36, pp. 48–49). While organisms causing sooty mould are associated directly or indirectly with attacks of scale insects, the Black blight organism is in no way associated with an insect attack. On the other hand however this fungus parasitises the leaf surface superficially.

The black covering is either in patches or extends all over the leaf area in the form of a crust. The crust is composed of dark brown (almost black) hyphae which grow in a radiating manner developing the fructifications also in the form of radiating discs each with a central mouth. The fungus attaches itself to the leaf surface by means of small cushions or hyphopodia, these latter also possibly aiding the parasite to withdraw nutriment from the host tissue. After a time the crust detaches itself from the leaf in the form of flakes.

Although a parasite, *Asterina camelliae* does no direct damage to the foliage and in this respect resembles the sooty mould organism that grows on the exudations of certain insects. Indirectly however, the fungal growth obstructs the easy access of sunlight to the leaf surface and attacked leaves are therefore naturally prevented from contributing their full quota of work in the vital process of carbon assimilation.

The effect of this interference might show itself after an extended period of attack and bushes might assume a debilitated or "shuck" appearance. The outward appearance of fields attacked by this fungus is considerably worse than the actual damage done.

On account of its comparatively harmless nature the disease could await disposal till the time of pruning. Where, however, there are indications of the disease spreading (as reported from some of the estates) spraying with a half strength Bordeaux mixture would effectively check the activities of the fungus.

Asterina camelliae has so far only been recorded from tea gardens in N.E. India, its occurrence in other tea growing countries not having been reported. It is likely that this fungus has existed on tea in South India at some previous period but it is probable that owing to the black covering it was associated with one of the sooty mould organisms.

Dimerium Wattii, Syd et Butl., a parasitic fungus on *Asterina camelliae* was invariably present on specimens of tea leaves attacked by the Black blight organism. In some of the specimens this parasite appeared to have entirely masked the presence of its fungal host.

Witches Broom:—Diseased leaf material that had been fixed in killing fluids show a greater preponderance of the greyish yellow bodies than healthy leaves similarly treated. In this respect nothing new has been added to what has already been reported in the previous Annual Report.

Bird's-Eye Spot (*Cercospora theae*):—An instance of severe leaf spotting due to this fungus was the cause of a query from one estate. The attack occurred on the very young leaves. This fungus however is present on practically all estates and is noticed mainly during the wet weather, but attacks have always been mild causing little or no damage.

Aspergillus *sp*:—Some tea leaf specimens received from an estate in the High Range were found attacked by a species of *Aspergillus*. The mycelial growth was of a yellow colour growing over either surface of the leaf. The plant accompanying the leaf sample had succumbed apparently as the result of some other cause. Damage to the leaves could not be attributed to the *Aspergillus* found on it, as the fungus was found entirely superficially. The perithecial fructifications were observed on the leaves embedded in the yellow mycelial clumps.

Stem Diseases:—No record of the occurrence of any serious stem disease was made during the year. The number of enquiries regarding this subject were very few. An instance of brown lesions on tipping shoots when examined disclosed the presence of a *Gleosporium* on the lesioned parts.

Wood Rot:—*Avoidance and Treatment.* In the last Annual Report (1936/37) under this head, an experiment undertaken on one of the station plots was described. During the period the experiment was under observation it was noticed that the treated branches were slow to recover from pruning as evidenced by the very few new buds that had sprouted on the treated surface. Also, where black paints had been employed the treated surfaces were in a large number of cases considerably damaged, the bark and the wood having been killed to some depth, with evidences of canker formation running along the entire length.

During the season under review the same experiment was laid out on another plot on a more rational basis to enable a statistical interpretation of the results. In the present experiment tar has been eliminated and an adhesive has been added to the lime in order to make the lime wash adhere for some time and prevent its being washed out as happened during the last season. So far this addition has proved to have been of some benefit. Although no appreciable number of cankers have yet been noticed, the treated surfaces (whether with white lead or some tar product) have been found damaged. The shooting capacity has also been adversely affected in the case of most of the paints except lime wash with adhesive.

So far as present observations indicate, the use of paints appears to cause damage to the bark and to a part of the wood also, and since therefore their application might lead to undesirable results they cannot be recommended. Lime washing with an adhesive might prove beneficial if this could be done without much expense, but the addition of an adhesive means an increase in the cost of application. Its protective value requires further investigation.

Fungus on dead tea branches:—The presence on dead Grevillea of an ascomycetous fungus *Tryblidiella rufula* and the probable presence of another species of the same fungus on tea branches were indicated last year. On a tea bush which was found attacked by *Botryodiplodia,* some of the branches of the stem were also found harbouring the *Tryblidiella.* Specimens bearing the fructification have been submitted to the Imperial Institute for identification.

It seems rather curious that the fungus, although occurring on dead shoots, should be so difficult to obtain among the large quantities of prunings that are

made available year after year at the time of pruning. On the other hand, this ascomycetous fungus has been observed occasionally developing on branches attacked by some other disease. The first specimens were found on branches previously infected by *Corticium salmonicolor* and, as already stated, the present specimens have been found on a bush that had died on account of *Botryodiplodia.* To what exactly this behaviour is due has not been clearly understood but it is thought that the fungus may prove to be a weak parasite.

Moss Cleaning:—Enquiries regarding method of dealing with "hide-bound" and moss-grown bushes have in recent times been frequent. The use of caustic washes as a preventive has met with encouragement and a suggestion has emanated as to the possibility of using this solution in the form of a spray. It is contended that spraying is cheaper and more effective than applying it with the aid of hessian or coir brushes. An attempt was made to determine the amount of liquid needed to spray an area possessing a known number of pruned bushes, with the result that as much as eighty gallons were found necessary to spray about 2,700 bushes. In determining the amount of liquid necessary it has to be borne in mind that the age of the bushes and the type of pruning adopted are factors that vary considerably. The bushes under experiment were about five years from planting and had not formed a full frame. Mature bushes would require a larger quantity of liquid and therefore the cost of application would vary. During the operations it is observed that a considerable quantity of the liquid is wasted while moving from bush to bush. It is also difficult to wet uniformly the entire frame—generally the upper surface is adequately moistened, but unless a special effort is made to position the lance and the nozzle almost at ground level turned upwards, the lower surfaces of branches as well as the collar might not receive the benefit of the application.

Against application with the aid of sprayers there is the method of applying the caustic wash (2%) with the aid of a coir brush by a labourer. This operative is able to cover the entire frame-work of each bush with a considerably smaller amount of the wash. The other advantage of hand application is the entire elimination of any capital outlay on spraying outfits and their renewals.

Root Diseases:—Enquiries regarding root diseases or their control were not as conspicuous as during the past seasons. Treatment of *Rosellinia* affected patches with bazaar alum has been undertaken on an estate in the Nilgiris. The result appears promising but the experiment is still under observation and is being watched with interest. Brown root-rot (*Fomes*) and Black root-rot (*Rosellinia*) have been the two most frequently reported root diseases during the year.

In nursery beds, young plants have been attacked by nematode worms and *Rhizoctonia bataticola.*

Shade, Green Manure and other Crops.

Grevillea.

Leaf Fall:—Grevilleas were found suffering from a leaf fall disease on some estates in the Nilgiri-Wynaad and the following are some of the details so far known regarding the causes and effect of the infection.

The first report of a Grevillea leaf disease was received in the early part of October, and this was followed by several other estates also reporting the existence of a disease causing defoliation of the youngest leaves. So far, the disease appears to have occurred only on some of the estates in the Nilgiri-Wynaad, the other districts not having reported any such occurrence. This limits its occurrence at present to elevations between 2,500 ft. and 3,500 ft.

Assuming that weather conditions at this period and in the district mentioned have been more or less comparable with those recorded at the Experimental Station, the following details are of interest. A heavy rainfall at this period (July) was followed immediately afterwards by a dry spell, which was in turn broken by a second shorter period of rain. From the presence of the large amount of bloom over the young leaves, it is considered likely that the annual leaf growth in Grevillea has most probably coincided with the extremely heavy rains experienced at this time, and it is probable that the young leaves have not been able to withstand this condition during their early development.

Symptoms. The most noticeable feature of the disease is a leaf fall from the growing shoot. The older leaves do not appear to be affected very much although numerous discoloured brownish spots are present denoting dead areas in these leaves. On the very young leaves the spots are not so prominent, but they show a curled-in effect, the affected areas being dead and brownish.

The shoots bearing the foliage are apparently in a sound condition though a die-back is reported to occur subsequently, but this requires confirmation. It may be that as a result of the defoliation, the shoots give a moribund appearance. Specimens examined have shown that the branches denuded of their foliage continue to be green with an unopened dormant bud, the latter covered with an abundance of reddish brown bloom (hairs). However, it has not been possible to determine if such a die back has followed defoliation on all the estates that have reported the disease.

Causal organism. Two fungal organisms have been found constantly in association with diseased foliage viz., a *Pestalozzia* sp. and a Phomaceous fungus *Phyllosticta.* It is at present not possible to determine which of these has been the real cause of damage, although, on account of its comparatively less damaging effect in the case of tea, it is possible the first of these organisms might be ruled out as unimportant. Even so, there are sufficient grounds to believe that *Phyllosticta* may also be an incidental agency that has found its way under certain climatic conditions. One cogent reason in support of the *Phyllosticta's* incidental nature is the presence of the fructifications of *Phyllosticta* on completely healthy foliage, young and old alike. At present the only reason that could be suggested for this fungus being the cause of damage is circumstantial in the shape of its constant association on young diseased leaves, but then its presence on healthy leaves casts doubt as to its parasitic rôle. In this connection it is of interest to note Petch's observation in the case of foliage of *Hevea* being attacked by probably a similar species of fungus. He is of the opinion that climatic factors rather than parasitic agencies are primarily responsible for the defoliation. In a like manner the *Phyllosticta* on the Grevilleas may be of a secondary nature.

In Ceylon, at about the same time as in S. India, defoliation in Grevilleas on lower elevations appears to have occurred (600–1500 ft.) but such defoliation has been mildly repeated five or six times during the year and this has led the Mycologist of the Tea Research Institute to feel that climatic conditions do not entirely explain the cause of damage. The suggestion has therefore been put forward that Grevilleas are perhaps not quite suited to lower elevations. The experience of South India in this regard is at present not known, except in the case of one estate in South Travancore, where all the evidence appears to indicate that climatic conditions do not favour the growth of Grevilleas on this estate.

Specimens of the leaves were sent to Dr. Gadd for comparison with the Ceylon specimens and confirmation as to the similarity of the fungus has been

received. A few samples have also been submitted to the Imperial Institute in London and their identification is awaited with interest. The Imperial Institute has confirmed our determination.

An obscure Grevillea Disease:—From an estate in Travancore specimens of Grevillea consisting of roots, stem, branches and leaves have been received, and these have been derived from what appear to be debilitated trees. Some of the root samples that were received here shew considerable malformed tissue in the form of burr-like outgrowths sometimes with numerous hollows, or merely a mass of compact tissue. Such outgrowths generally occurred where a wound affecting the bark and part of the wood was present. It was however not known whether these malformed tissues were a common feature of all debilitated trees. Examination of the root tissue did not show any serious pathogenic organisms except in one or two small rootlets, in these latter the fungus *Botryodiplodia* being present.

The trunk of the tree was free from fungal attack but the branches exhibited large numbers of cankered tissues, the bark being dead and black in colour. The wood however showed no indication of being diseased but continued to be healthy.

The leaves were all dry and bore on their surface fructifications of the fungus *Phyllosticta* and in this respect resembled the Leaf Fall disease discussed earlier.

On the basis of the evidence available the disease could not be attributed to attacks of *Botryodiplodia.* The number of root branches so affected were very few, only on two very small rootlets was the discoloration evident and these have under moist chamber conditions produced the fructifications of *Botryodiplodia.* It is considered that this fungus, on the analogy of the disease in tea, is again an indication of the existence of some debilitating cause rather than the cause itself.

The fissured bark surface is probably the result of the branches having been weather-beaten.

The role of *Phyllosticta* in causing defoliation has been discussed earlier and its presence in the present instance might, as in the other cases, be incidental to the occurrence of a certain set of climatic factors. Data as to how long the damage has been noticed, the age of the trees, the elevation of the estate, are wanting.

Root diseases:—*Ustulina* (Charcoal Stump Rot) *Fomes* (Brown Root Rot) and *Poria* (Red Root Rot) were reported on specimens of Grevillea roots received for examination.

Acacia decurrens.

Considerable damage was reported to have occurred to the foliage of Acacia decurrens consequent to attacks of *Cercosporella theae.*

The same trees later on during the drier months, developed various symptoms and a number of these trees were subsequently reported to have died. Examination of the trunks and branches indicated reddish pycnidia of a still undetermined fungus. Stylonectria but because of the different asci in some of the groups, it is suggested it may be a Nectria. However, because of there having been no asci in the specimens examined, it may be named Stylonectria. The roots of these trees shew the existence of two distinct organisms—the sclerotial fungus *Rhizoctonia bataticola* and another basidio-mycetous fungus producing orange mycelial strands on the surface of affected roots. The identification of this basidio-mycetous fungus has not been made because of the absence of any fruiting body, but the Manager of the estate has kindly offered to co-operate and allow a few of the dead trees to remain in the soil in order to observe whether a fructification will develop and so enable the organisms to be identified. As

yet no report is available but it is hoped that this experiment will meet with success.

Crotalaria anagyroides.

The black leaf disease (*Parodiella sp.*) on Crotalaria anagyroides was reported on specimens from an estate. From the same estate tea leaves attacked by Black blight (*Asterina camelliae*) were also received. It must be stated here that these diseases although causing a general blackening of the foliage have nothing else in common, being caused by distinctly separate fungi.

Cardamom Leaf and Root Diseases.

Very little is yet known of the diseases of Cardamoms in South India. Apart from a "Mosaic" disease that occurs in the Anamallais two other diseases viz., a "Rhizome rot" and a "Bud rot" have been noticed during the season under report. The information given here is based on the specimens received and does not perhaps present as complete a picture as desirable. The following notes are given in view of their possible interest to Cardamom Planters.

The "Rhizome Rot":—Healthy rhizomes or stools from which the plants are raised show an ivory white appearance and the leaf shoots when appearing show the bud as a small greenish protuberance; neither the white colour nor the greenish bud is however apparent when the rhizome is diseased. Instead of the healthy appearance the rhizome becomes slightly shrivelled and a brown discoloration extends all over the rhizome tissue.

Examination of the material has shown the presence of a fungus (*Rhizoctonia solani*) and also eelworms probably of the genus (*Anguillulina.* Another fungus *Pythium* also seems to have been obtained from the diseased parts but, in the specimens received here, this organism was not to be found. As a result of attack the stools appear to exhibit a "damping off" effect with the larger part of the aerial growth collapsing at ground level. Of the organisms mentioned as having been observed all the three are perhaps capable of bringing about the so called "damping off", although no inoculation experiments have been carried out on Cardamom plants themselves.

With a plant like Cardamom, it is rather difficult to suggest any easy means of control. The removal of all discoloured rhizomes and destroying them would not by itself eliminate all the infected material as the organisms concerned are able to live for a time in the soil. Moreover, to eliminate entirely the diseased material might not be a practical measure since it requires pulling out the entire stool a major part of which continues to remain healthy. In diseases of the kind here mentioned stress is generally laid on the use of disease-free material and on soil drainage to remove excessive moisture.

"Bud Rot" of Cardamom Leaves:—The exact cause of the disease is still a matter of doubt although from the evidence available, there is strong reason to suspect the association of a nematode worm in bringing about the damage to the unopened leaf buds of Cardamoms. Certain fungal organisms, notably a sclerotial fungus resembling *Rhizoctonia solani* and a species of *Gleosporium* were also found on the diseased leaves, but the very large number of nematodes actively moving about together with their eggs laid on the leaf buds, strongly suggests the disease to be of nematode origin.

Maggots rolled in the leaf were also of common occurrence but no importance is at present attached to these in the causation of the disease. The flies

emerging from the maggots have been of a miscellaneous sort considered to be of a scavenging type.

The disease, as at present known, is confined to the unopened leaf bud and is reported to attack only a half of the bud, the other, more interior, half remaining unaffected. The bud opens out showing tattered parts of the damaged half still adhering to the midrib. Small translucent spots on the upper surface of the leaf bud are the first signs of the disease. The translucent spot gradually enlarges resulting in a sort of wet rot engulfing the linear half of the bud from the tip downwards. When however the climatic conditions are adverse for the disease, (the disease being prevalent only during wet weather) the extension of the rot decreases, the entire leaf then showing only a small diseased area on opening out.

The older leaves remain unaffected and are therefore of a healthy appearance, and the same seems to be true of the unattacked half and those leaves enlarging during dry weather.

Based on the symptoms described above and the presence of a large number of worms, an attempt was made to correlate the disease with the worms by obtaining leaf buds in all stages of the disease together with a few healthy buds. These specimens were maintained under damp conditions and were under observation till the buds were completely affected. One striking feature that stood out in contrast to the observation of the Manager was that in the specimens maintained in the laboratory the entire leaf bud was attacked and not only a half of it.

Examination of the leaf bud showing only translucent spots disclosed small punctures on the interior of the leaf surface, i.e., on the upper surface of the leaf and, when mounted and examined, some free nematode worms were found on the tissue in an active condition. The number of such translucent spots varied considerably. On unrolling the bud, the more interiorly placed parts of the lamina were found comparatively less affected, as evidenced by the fewer number of the spots that were apparent, than on the outer layers of the lamina.

Leaf buds more heavily attacked showed not only the actively moving worms but also eggs laid on the surface of the leaf. Such specimens shew worms of both sexes and, in appearance, the males resembled the female worms except for the uvular opening which was situated more or less in the centre of the body in the form of a cleft. In some of the specimens a second kind of worm was discernible, these worms having curved string-like structures at their tail ends, but since only a small number of this kind of nematode were to be seen in the specimens it is considered that these may not be of much importance in the causation of the disease. The more numerous worms had an eel-like body tapering towards the tail end and are tentatively considered to belong to the genus *Aguillulina*. Specimens of the material have been forwarded to the authorities of the Imperial Bureau of Agricultural Parasitology who are being kind enough to assist in the exact identification. Three different types of worms have been reported, viz., 1. Rhabditis. 2. Aphalanchoides. 3. Cephalobus.

The leaves and buds under the damp chamber condition during the progress of infection turned flabby and slimy, and an increase in the number of nematodes was always noticeable in such specimens.

The reasons that lead to only a half of every bud being attacked under natural conditions are not sufficiently understood but it may be possible that (1) as the leaf bud unfolds less protection is offered by the exposed leaf, or (2) a sufficient amount of free moisture (for the movements of the worms) may not be available as the buds unroll and open out the blades, or (3) the leaves on their

unrolling are sufficiently mature and hard as not to be palatable to the parasite, the thicker texture of the mature leaf probably rendering it more resistant. These are some of the possibilities, but there may be other more potent reasons in addition which it was not possible to determine under laboratory conditions owing to the lack of live plants.

The mitigation and, finally, the disappearance of the damage during dry weather may be due to lack of sufficient moisture (owing to the very rapid evaporation from the surfaces of leaves at this season) for the worms actively to participate in the destruction of the leaf bud.

There is one point that requires further elucidation, viz., the source of infection. It may be that the attack starts early in the life of the plant just when the first shoots are coming up from the soil surface, in which case presumably the worms are present in the debris on the soil or in the soil itself and migrate towards the leaf buds. A point of importance that requires further investigation is how subsequent infection takes place in the mature plant after a long interval of dry weather. Two possibilities may be suggested neither however having received confirmation, viz., (1) the hibernation may be effected by the production of cysts, (2) the worms may be living on debris and, with the advent of favourable weather, once again commence their ascent towards the bud, thus living as facultative organisms—a saprophytic life during dry weather and a parasitic existence during wet weather.

The only reason that has prompted the Department to suggest the possibility of the disease being of nematode origin is the invariable association of this organism in very large numbers in the tissues of the diseased buds. It is improbable that their numbers could be accidental and restricted to specimens reviewed at the Station. Beyond this it is at present not possible to determine the precise cause of the damage. Neither infection studies nor examination under field conditions have been made to determine finally the nature of the cause and such a study seems highly desirable.

Control measures at the present state of our knowledge of the disease should therefore be of a very tentative nature. On the assumption, however, that the disease is of nematode origin the following suggestions have been made:—(1) Remove all infected buds by cutting back and transfer them immediately to a container with a disinfectant. This operation would not only remove the infected bud, thereby affording a better chance for the appearance of a healthy leaf, but its immediate transfer to a disinfectant prevents the infective material from becoming distributed and remaining a potential source of fresh infection. (2) The application of a repellent to the plants treated as above. The use of lime sulphur solution or a dust might be given a trial, but no definite recommendation is possible without adequate trials. (3) Destroy by burning all rotting Cardamom leaves and twigs in the field.

Much still requires to be done before a more definite view can be expressed regarding this disease, the views and identifications of the authorities on the subject should form the basis of further investigation and a reply is awaited on this subject, all the available material having been submitted to them. None of the nematodes appear to be primarily parasitic.

BACTERIOLOGICAL

Bacterial flora in finished composts:—Three different bacterial organisms were isolated from samples of finished composts, all three showing considerable morphological and physiological differences. All the three organisms were, however, non-motile and non-spore forming rods, but of considerable variation in

their sizes. This difference in size was maintained under different conditions and indicates their being different organisms. For convenience these have been designated Bacteria "A", "B" and "C".

The following physiological differences were noticed in the three strains.

(1) *Gelatine liquifaction*:—Bacterium "A" when grown on Lemcogelatin indicated violent gas production but the first indication of liquifaction was only apparent after nearly four weeks. Bact. "B" caused a depression on the surface and liquifaction was complete at the end of the 2nd week but no gas formation was evident. Bact. "C", the first indication of gas formation and liquifaction was noticed on the third day and at the end of the week the entire medium has been liquified.

(2) *Litmus milk*:—Bact. "A" turned litmus milk alkaline, but with the lapse of time, the blue colour was found confined to the upper layer with a white opacity in the lower strata. In Bact. "B" a blue colour was perceptible but gradually the milk turned buff and a light tea coloured precipitation of a creamy substance was noticed at the end of the third week. Bact. "C" No blue colouration was observed at any time but gas formation occurred at surface region, the liquid became yellowish and translucent at top with a creamy precipitate below.

(3) *Uschinsky's Solution.* Bact. "A" produced at first a slight cloudiness on the 3rd day with the medium gradually turning greenish. Scum formation occurred but the liquid remained non-viscid. Bact. "B", cloudiness was followed by a white colouration and slight viscidity. Bact. "C", scum formation with a green colour and slight viscidity.

(4) *Colin's Solution.* There was no indication of growth in this medium of either Bact. "A" or "C". Bact. "B" however developed copiously with a pellicular growth on top and cloudiness.

(5) *Nutrient broth.* Bact. "A", feeble growth, with slight cloudiness, Bact. "B" developed a scum during the middle of the second week. Bact. "C", copious growth, with scum formation and cloudiness, and at the end of ten days the solution showed a reddish colouration.

(6) *Lemco Agar.* Bact. "A", slight liquifaction with an echinate growth, rapid gas formation. Bact. "B", slight liquifaction with a dirty white filiform growth. Bact. "C", filiform growth on surface with gas formation but not so violently as in "A". On slants slight liquifaction occurred.

Cellulose decomposition:—Although it was not possible to obtain decomposition of cellulose after the three bacteria had been isolated, in the still unisolated condition cellulose decomposition occurred under cultural conditions, a filter paper introduced being completely broken down. Gas production was apparent in the form of large bubbles which formed after being shaken at intervals.

Between two layers of compost a circular piece of filter paper was introduced and the dish was kept watered to keep the compost moist. It was found at the end of nearly a month that not a vestige of the filter paper was present, it having been decomposed. This is taken as illustrating the fact that the organisms causing destruction of cellulose persist even in a finished compost becoming active under favourable conditions.

In addition to these bacteria, two fungal organisms a *Rhizoctonia* and an *ascomycetous* fungus with rose coloured perithecia were obtained, but the latter could not be induced to fructify on agar media.

APPENDIX I.

ENZYMES OF TEA

(from thesis submitted by Dr. K. B. W. Jones for Ph.D. degree).

History of Investigations of Enzymes in tea:—The discovery of enzymes in tea dates from 1900. Bamber in Ceylon and Nanninga in Java appeared to have suspected their presence at about the same date, 1893, but it was not until 1900 that Bamber first isolated from fresh leaf a material possessing enzyme activity. This was independently confirmed by Aso, in Japan, in the following year.

Newton, in Ceylon, in 1901, evolved a histological method for determining the distribution of enzyme in the leaf, using tincture of guaiacum as a qualitative reagent. He found maximum development of a blue colour in the epidermis and in the vascular bundles.

Mann isolated an enzyme (sic) by macerating fresh leaf, or air dried leaf after moistening, with hide powder, squeezing out the resultant juice, precipitating by alcohol, and purifying by repeated re-solution in water and re-precipitation with alcohol. He obtained a white powder after drying at room temperature.

To determine the relative quantity of the enzyme Mann also used tincture of guaiacum. The degree of intensity of the blue colour, that this gave with the enzyme, was compared with that produced in a solution containing a known quantity of the ferment.

Mann found that the enzyme could be divided into two parts of which one gave a blue colour with the re-agent alone, while the other only gave a colour if oxygenated water or hydrogen peroxide were also present.

Bernard and Welter, in Java, in 1911, found it impossible to free the isolated enzyme (sic) from tannin. They found that the elements, Nitrogen, Sulphur, Phosphorus, Manganese, Magnesium, Potassium, Carbon, Oxygen and Hydrogen were always present. Deuss later found iron as well.

Bernard and Welter classified the two types of enzymes as oxidase and peroxidase. They found the oxidase of varying activity but the peroxidase activity was always intense.

The effect of chemicals and of temperature on the reaction with guaiacum was studied by Mann, and by Bernard and Welter. As regards temperature the results were as follows:—

TABLE I

	Low Limiting Temperature	Optimum Temperature	Maximum Limiting Temperature
Mann	. .	130° F	150° F
Bernard and Welter . .	32° F—50° F	115° F	165°F—212° F

Mann studied the effect of enzymes on the quality of tea, etc. He found that both the active enzyme (oxidase) and the total enzyme (oxidase plus peroxidase) decreased in passing from the bud to the second leaf and increased in the stalk.

He also found, by comparing the enzyme activity in leaf from varying estates in the Darjeeling district, that leaf which produced the most flavoury tea also contained the greatest quantity of these enzymes. He concluded that there was a relation between the quantity of enzyme and the quality of the tea.

Mann also found an increase in enzyme activity during withering and evolved the following table for the time required for the enzyme content of withered leaf to reach a maximum.

TABLE II

Correct Duration of wither according to Mann.

Average Mean Temperature	Very Dry Weather or with Wind	Ordinary Withering Weather	Atmosphere Saturated
80°F	19 hours	19½ hours	26½ hours
81°F	18 ,,	18½ ,,	25 ,,
82°F	17 ,,	17½ ,,	25¼ ,,
83°F	16 ,,	16½ ,,	22½ ,,
84°F	15 ,,	15½ ,,	21¼ ,,
85°F	14 ,,	14½ ,,	20 ,,

Finally, Mann associated the phosphoric acid content of soil with the quantity of enzyme in the leaf, and quality of the tea produced.

Bamber disagreed with Mann that the quantity of enzyme influenced the flavour of the resultant tea and cited the facts that flavour was best at high elevations and during periods of low temperature, when enzyme activity would be at a minimum, and that he could find no variation in enzyme content during the pruning cycle.

Later experiments in Java showed no correlation between the quality of the tea, the amount of enzyme present, or the amount of iron, manganese or phosphorus.

It will be noted that whereas the presence of enzymes was recognised, authorities differed as to the significance of their effects.

In 1932 Shaw and Jones (Theotannin in relation to Black Tea) put forward a hypothesis that one of the changes occurring during withering was the formation of a synthetic peroxidase system consisting of a metallic hydrosol of manganese or iron, and a hydrophilic colloid, theotannin.

Shaw (Annual Report of the U.P.A.S.I. Tea Scientific Department 1932–33) commented on the fact that the previous investigators on enzymes had all been working on crude, water soluble pectin. Shaw found that after completely extracting leaf with alcohol, extracting the pectic substances from the residue with 40 per cent. alcohol, and precipitating the filtrate with 70% alcohol, the

filtrate contained a substance which could be isolated by evaporating to small bulk and adding a large excess of absolute alcohol. By carefully avoiding the presence of water it was possible to wash, filter and dry this material and obtain a nearly white powder from green leaf or a slightly more coloured variety from made tea.

This substance is still of doubtful constitution, but Shaw showed that it contained about 20 per cent. of ash, about 40 per cent. of a tannin body and about 2 per cent. of non-caffeine nitrogen. About 40 per cent. is at present unaccounted for. The ash contained phosphoric acid, magnesium, potassium, manganese and sulphate.

In physical properties this substance closely resembles an alleged glucoside isolated by Nanninga and which he thought was connected in some way with the aroma of tea.

Proceeding to an investigation of the precipitated pectin, Shaw found that this contained about 20 per cent. of ash; 40 per cent. of pectin, calculated from the calcium pectate equivalent; and about 20 per cent. of a remainder; and he concluded that "it would appear that the essential difference between the pectin samples, and the new substance, (above) is that in the pectin samples tetramethyl-pectic acid takes the place of the theotannin in the new substance".

In further work on pectin, Shaw (Studies on the Pectic Substances in Tea) concluded that the unknown remainder was a substance closely resembling nucleic acid, in combination with metals.

Taking into consideration the fact that previous investigators never could obtain their enzyme free from nitrogen, or from tannin, and the mineral constituents found by Shaw in his two new substances, it appears likely that one or both of these substances is intimately related to the enzyme of tea. Pectin itself is not likely to have enzymatic properties.

Preliminary work on Enzyme Activity:—

Since enzymes play a considerable part in fermentation it was desirable to enquire further into their action.

It was considered that the isolation of pectin samples, or the two more difficultly isolated substances of Shaw, was not well adapted to the investigation in view. With such labour and expense it might be demonstrated that these substances gave a colour reaction with tincture of guaiacum but there was no prospect that such a method of attack would give results suitable for application to factory problems as quickly as was desirable.

An alternative proposal was a study of the effect of solutions containing enzymes on tannin solutions containing no enzymes, i.e., to use an inoculation technique.

The first experiments were directed towards obtaining material suitable for use as an inoculant. Solutions containing enzymes could be obtained by infusing green leaf or withered leaf but obviously only a limited application of heat could be permitted. The quantity of enzyme extracted however would probably be very small and hence its effect might be difficult to estimate accurately. Squeezed out extracts of green or withered leaf were considered more satisfactory. Tests were made on the extracts obtained by submitting small quantities of leaf to a pressure of about 400 to 500 lbs. per square inch in a miniature hydraulic press.

TABLE III

	Weight of leaf	Volume of Expressed juice	Iodimetric Totals Equivalent N/20 Thio per c.c.
Green Leaf	50 grams	10 c.c.	..
60% Withered Leaf ..	50 ,,	8 c.c.	..
Green Leaf	50 ,,	12 c.c.	17.5 c.c.
55% Withered Leaf ..	50 ,,	7 c.c.	42.5 c.c.

For a trial inoculation, green leaf was withered to about 62% in 24 hours, 50 grams of leaf were expressed and 5 c.c. of juice was obtained. This was very dark in colour but clear. A theotannin solution was prepared containing about 0.65 gms., per 150 c.c. Mixtures were made up as follows:—

A 2 c.c. Extract. + 75 c.c. Water.
B 75 c.c. Theotannin solution + 2 c.c. Water.
C 75 c.c. Theotannin solution + 2 c.c. Extract.

These were placed in beakers of similar size and shape. The iodimetric totals equivalents of 5 c.c. of each mixture were estimated at intervals.

A + B — C was a measure of the effect of enzyme action if any.

Results were as follows:—

TABLE IV

Time	A	B	A + B	C	A + B — C	Percentage effect of Enzyme action
0 Hours	3.05	19.8	22.85	22·75	0.1	0.4 %
2 ,,	2·9	19.9	22.8	22.15	0.65	2.8 %
8½ ,,	2.8	19.8	22.6	21.85	0.75	3.3 %
20 ,,	2.6	19.8	22.4	21.35	1.05	4.6 %
25 ,,	2.6	19.9	22.5	21.5	1.0	4.4 %

From the first result it is evident that a small error in titration makes a significant difference to the result. Nevertheless, plotting time against percentage effect, very little smoothing is required to produce a satisfactory curve. This initial experiment was important as it showed that it was possible to estimate enzyme effects iodimetrically. Shaw's iodimetric method has therefore been employed throughout this work.

At the same time the action was relatively slow and the effect produced small. Moreover, the effects observed are not comparable with practical conditions of tea manufacture since concentrations were infinitely too small and the conditions of availability of air were very different to those prevailing in practice.

The experiment also showed that dilute theotannin solutions were not greatly affected by oxygen in the absence of an enzyme but could be activated by the addition of the enzyme, although only in a leaf extract.

Search was made for a convenient method of applying the idea of inoculation in a form more in agreement with factory conditions. It was established that green leaf infusions could be substituted for the theotannin solution. The heating of the infusion during the extraction period left a solution which contained tannin bodies but had no marked tendency for spontaneous oxygenation.

Experiments were then made with dried green leaf and it was found that while desiccator dried leaf could be preserved for some time, after moistening with water and pulverising the leaf it fermented readily. Oven dried leaf would not ferment when moistened with water but it appeared that fermentation could be induced in it by moistening with an extract from fresh or withered leaf. Moreover, such fermentation appeared to be complete in a period of five or six hours which compares well with actual practice.

It was also found that the use of the hydraulic press was unnecessary. Its use was attended by difficulty as it could only take a small quantity of leaf and a considerable proportion of the juice was wasted, while with withered leaf it was difficult to obtain more than 2 or 3 c.c. of extract without using so high a pressure that the filter cloth used was liable to burst.

A simple method of greater efficacy was to pass the leaf through a mincing machine and squeeze by hand through a cloth. Larger quantities of leaf were required, and with hard withered leaf it was necessary to add a proportion of water to the minced leaf, allow to soak for a few minutes and then squeeze out the juice. The resultant extract was very thick and turbid but could be filtered through fine muslin without pressure, and the filtrate could be measured out in aliquot portions by a graduated pipette.

Procedure Adopted

A. *Preparation of Extract*:—The material employed was minced, the product was roughly weighed, and water was added sufficient to bring its moisture content up to that of fresh leaf or to some other convenient and appropriate standard. The mixture was stirred and kneaded until all the water appeared to be absorbed into the tissue of the leaf. The mass of leaf was then placed in a strong cloth and squeezed by hand until as much juice as possible had been expressed. This was then filtered quickly through fine muslin to remove any macroscopic particles of leaf. The filtrate could then be used for inoculation purposes.

In some experiments the enzyme extract was to be derived from dry or nearly dry leaf. In such cases the leaf was broken up while dry, water was added to it in a powdered state and the material was put through the mincing machine after it had absorbed water.

Care was taken not to force the leaf through the mincing machine too rapidly as a considerable rise in temperature could be induced by using too much force and this might in some cases have undesirable effects.

TABLE V

Sample	% Water	Weight of minced leaf gms.	Weight of water added gms.	Volume of Extract obtained c.c.	Thio Value of 20 c.c. of Solution c.c. N/20	Water content of squezed leaf %	Thio Value of Total Extract c.c. N/20
A ..	100	73	..	18	8.8	64	790
B ..	91	73	..	18	9.1	61	820
C ..	91	73	7	23	8.4	62	965
D ..	75	54	..	9	12.7	56	570
E ..	75	52	17	22	8.7	60	955
F ..	52	33	16	14	9.5	51	665
G ..	54	34	34	27	6.2	58	835
H ..	52	33	16	11	9.3	55	810
I ..	52	33	33	26	8.75	57	1135

Before dealing with the comparative enzyme activity of leaf samples of varying water content an experiment was carried out to determine the amount of water it was necessary to add in order to obtain extracts of approximately the same iodimetric reducing power.

Nine 100 grams samples of green leaf were weighed out, and eight were allowed to wither. At intervals the percentage wither was noted, and extracts were obtained from the samples after they had been minced. 5 c.c. of each extract were pipetted into about 200 c.c. boiling water, boiled for about 5 to 10 minutes under a reflux air condenser and the solution made up to 500 c.c. These solutions were estimated for iodimetric totals, (Shaw, Theotannin, Part I). The following table gives details of the various extracts and their iodimetric equivalents. Table V.

Samples C, E, G and I received enough water to bring them up to their original moisture content. Samples F and H only obtained half this quantity of water. For C, E, F and G the material absorbed the excess water almost immediately but H and I were left to soak for 30 minutes.

The quantity of minced leaf varied since delivery from the machine was not quantitative and a certain wastage occurred each time.

This table of results gives sufficient information to enable one to obtain extracts of approximately the same strength from leaf samples with varying water content.

It should be noted that the strength of these extracts is judged by their iodimetric totals value and this does not necessarily vary proportionately to the enzyme content of the extract. Nevertheless it is a basis of comparison which can be successfully employed in some cases.

B. *Preparation of Medium to be inoculated*:—Green Leaf is dried in a steam oven to constant weight. It is then powdered and sifted through a sieve (60 meshes per inch). Material which does not pass through is again pulverised and resifted. The coarse dust obtained is then passed over a finer sieve (90 mesh) and the fine dust coming through is rejected. In this way dried leaf of approximately uniform particle size is obtained. This is again dried for half-an-hour and transferred to a clean dry bottle which has been well washed with strong acid. This bottle is then tightly stoppered and kept in the dark until required.

Leaf treated in this way may be kept for a fortnight or more without noticeable deterioration. After keeping for a longer period it tends to become discoloured where it touches the glass surface, and the iodimetric equivalents of infusions of the leaf tend to decrease slowly.

For inoculation purposes 1.5–2.0 grams of this leaf are used.

Tests were applied to find the magnitude of sampling error and experimental error in the preparation and estimation of infusions and also to determine whether any effects, determinable iodimetrically, were produced if the leaf was moistened.

Four 1.5 grams samples of dried leaf were weighed out. One was infused immediately. The remainder were moistened with 3 c.c. of water and allowed to stand in small dishes for periods of 2, 4 and 6 hours respectively before being infused. The infusions were prepared by transferring to a litre boiling flask containing about 425 c.c. of water, simmering for 45 minutes under a reflux air condenser, transferring while hot to a 500 c.c. graduated flask, adding washings of the boiling flask, cooling and diluting to the mark. The leaf particles were not removed at this stage. When the infusion was required for estimation it

was first filtered through a Whatman No. 4 filter paper, rejecting the first few c.c. of filtrate.

The above infusions were estimated for iodimetric totals and for the normal salt-gelatine precipitation equivalent using a 15 minutes reaction period and N/20 Thio for titrating. (Shaw, Theotannin Part I). Results were as follows:—

TABLE VI

Sample	Iodimetric Equivalents in terms of N/20 Thio per 20 c.c. of infusion		
	Totals	Filtrate	Precipitation
1 ..	17.95	4.45	13.5
2 ..	18.1	4.55	13.55
3 ..	17.9	4.55	13.35
4 ..	18.1	4.6	13.5

The results show that the total values and precipitation values are constant to within small limits of experimental error.

C. *Method of Inoculation*:—This varies somewhat according to the particular point being investigated. The general outline is to weigh out the dried leaf into a small petri dish, moisten this with extract added from a graduated pipette, and to stir the mixture with a glass rod until the liquid has been uniformly distributed and absorbed. The fermenting leaf is then covered with a wet filter paper, or some other convenient arrangement is made, to prevent the mixture drying out, without unduly limiting the air supply.

THE EFFECT OF TIME ON ENZYME ACTIVITY

Experimental Procedure:—Five 1.5 gm. samples of powdered dried leaf were weighed out into dishes, and 15–20 c.c. of extract were obtained from a sample of withered leaf. 2.5 c.c. of the extract were added to four samples of dried leaf and fermentation was allowed to proceed for 1, 2½, 4 and 5½ hours respectively. At the end of these periods the samples were infused, and in due course estimated for iodimetric totals and precipitation equivalent. Check values were obtained for the untreated samples of dried leaf and for the withered leaf extract at zero time.

TABLE VII

Sample	Time hrs.	Totals equivalent in c.c. N/20 Thio	Enzyme effect in c.c. N/20 Thio
A ..	0	25.55	0
B ..	1	23.4	2.15
C ..	2½	22.2	3.35
D ..	4	21.35	4.2
E ..	5½	20.65	4.9

By plotting the change produced by the enzyme against time, a logarithmic curve is obtained, and by plotting the change against log time the curve obtained is practically a straight line curve. This is in conformity with expectation in respect of an enzyme action.

Results of the estimations of the iodimetric precipitation equivalents showed that the greatest variation in the precipitation value was only 0.35 c.c. representing a very small percentage of the total change. This also was in agreement with expectation and tends to show that in this respect the fermentation induced by the inoculation of dried leaf with a withered leaf extract is similar to that which occurs in normal practice.

A significant point in relation to the practical procedure is that fermentation takes place most rapidly at the beginning.

The Effect of Temperature on Enzyme Activity

Five samples of powdered dried leaf, each of 1.8 gms., were weighed out, and four of these were placed in 100 c.c. conical flasks and immersed in baths kept at different temperatures. To each, 2.5 c.c. of an extract of withered leaf was added and the reaction was allowed to proceed for four hours, after which the contents of the flask were infused in the ordinary way and estimations of the iodimetric totals equivalent were carried out. A check value was obtained for the dried leaf itself plus the withered leaf extract.

Temperature control was effected, in the case of the two determinations below room temperature, by adding small quantities of freshly prepared ammonium nitrate solution to the surrounding bath, and in the case of the two determinations above room temperature by the use of a small spirit lamp applied at frequent intervals as required. In this way temperatures were maintained at 60½° F to 61½° F, 68½° F to 69½° F, 79½° F to 81½° F, and 87½° F to 90½° F.

Results:—

Table VIII

Sample	Temperature	Totals Equivalent in c.c. N/20 Thio	Enzyme Effect c.c. N/20 Thio
A ..	..	25.2	0
B ..	61° F	23.85	1.35
C ..	69° F	23.4	1.8
D ..	80½° F	22.75	2.45
E ..	89° F	21.7	3.5

Discussion:—For the temperature range 60°F to 80°F the change is proportional to the change in temperature but between 80°F and 90°F the change is accelerated.

The variation when the temperature approached 90°F was not unexpected since Mann had previously found that above 85°F there was a rapid decrease in tannin, as shown by his method of analysis.

Further investigations on this point, including estimations of the precipitation and methylene equivalents, would probably throw more light on this subject which is of practical importance since the temperature of the leaf during rolling sometimes reaches this region.

The results of the above experiment are interesting in comparison with work by Nanninga in 1902, (Technologie due The, by Neuville 1926). Nanninga carried out qualitative experiments on leaf dried in a desiccator, powdered, and subsequently moistened when required. He found that fermentation was exceedingly slow below 20°C, and rapid but unpleasant in effect at 30°C or over. (cf. 58°F and 86°F). Now in practice South Indian estates at an elevation of 5000 feet or more often carry out fermentation at this low temperature and find that the fermentation although slow is not nearly as slow as would be expected. There is, in fact, very little difference in average duration of fermentation at low and at high elevations.

In order to explain this apparent incompatibility between laboratory experiment and factory practice the suggestion is advanced that the activity of the enzyme at low temperatures depends to some extent on the temperature at which the leaf is grown. The leaf used by Nanninga was probably grown at a temperature of about 75°F to 80°F while the leaf used in the quantitative experiment quoted above was grown at about 70°F. The truth of this hypothesis could be tested by repetitions of the experiment in the months of April and December when the average mean temperatures in this district are 76°F and 69°F respectively. It is possible that the source of such a variability in response to temperature might be traced to either a change in the nature of the enzyme or to the variation in theotannin which probably occurs.

This point is of practical importance as the majority of planters consider it advisable to maintain the fermenting rooms as nearly as possible at some constant temperature, and practical evidence as to the best temperature is conflicting. On the basis of the above hypothesis, it is possible that the rule to be followed in practice may be to maintain the fermenting room at the average mean temperature of the external air. Many further experiments will be necessary before a full statement can be made concerning the effect of temperature on enzyme activity in practical tea manufacture, but the means of obtaining the information required, by a quantitative method is now available.

The Effect of Concentration of the Enzyme on the Fermentation of Tea

Introduction:—In studying the enzyme action on theotannin, with respect to the effect of concentration, it is desirable to recall that in practical tea manufacture the quantity of theotannin present is probably more constant than the quantity of enzyme. Shaw showed that the theotannin content of green leaf could be calculated from the dry weight, and applying this to normal factory conditions one is led to the conclusion that a wither, which is considered satisfactory by planters, results in a concentration of theotannin in withered leaf equivalent to one gram of tannin to between five and six grams of remaining water.

Mann found that the enzyme content of green leaf varied considerably. Quantitative experiments showed more than twice as much enzyme in one type of leaf as compared with another. Moreover the activity of the enzyme or its amount vary appreciably during its withering.

TABLE IX

Totals of Equivalents in c.c. N/20 Thio.			Enzyme effect	Enzyme effect per totals of 20 c.c.	Relative enzyme Concentration
D.L. W.L. Col. 1	W.L. Col. 2	D.L. Col. 3	Col. 4	Col. 5	Col. 6
EXPERIMENT I.			*Temperature* 76°F		*Duration* 5½ *hours.*
18.15	0.00	18.15	0.00	0.00	0.00
17.55	1.45	16.10	2.05	2.20	0.20
17.35	2.80	14.55	3.60	3.95	0.40
17.85	4.15	13.70	4.45	4.90	0.60
18.45	5.45	13.00	5.15	5.65	0.80
19.25	6.70	12.55	5.60	6.15	1.00
EXPERIMENT II			*Temperature* 88°F		*Duration* 4 *hours.*
20.00	0.00	20.20		0.00	0.00
18.95	0.90	18.05		1.95	0.20
19.00	1.70	17.30		2.70	0.40
19.25	2.50	16.75		3.25	0.60
19.75	3.30	16.45		3.55	0.80
20.25	4.10	16.15		3.85	1.00

It is therefore probable that in practice the concentration of enzyme in the leaf at the beginning of fermentation is much more variable than the concentration of theotannin.

Accordingly in the two experiments to be described, in any given series of estimations the tannin content has been maintained as constant as possible while the concentration of the enzyme has been varied.

The results of these experiments are given in one table for convenience but notes on the two different series are given first.

Experimental Procedure:—In Experiment I 1.5 grams of dried leaf were employed for each inoculation. To these, varying quantitatives of withered leaf extract and water were added so that a total value of 2.5 c.c. was added to each, the quantity of extract being increased from 0.5 c.c. by steps of 0.5 c.c. to 2.5 c.c. the water added being correspondingly diminished from 2.0 c.c. to 0 c.c. Check infusions were prepared of the dried leaf alone and of the withered leaf extract before and after the period of enzyme activity. The temperature was kept at 76° F for 5½ hours.

In Experiment II, 1.6 grams of dried leaf were used, but otherwise the procedure was similar. The temperature was kept at 88° F for four hours.

Results:—

These are given in an appended table. In Column 1 is given the observed iodimetric totals value of 20 c.c. of each infusion of leaf after fermentation. Column 2 gives the totals value of the withered leaf extract contributing to the figure in Column 1; this value being calculated by interpolation from the original and final checks on the withered leaf extract. Column 3 is the difference between the two figures and indicates the totals value of the dried leaf after fermentation. Column 4 gives the change in totals value due to the effect of the enzyme on the dried leaf.

In column 5 this enzyme effect has been recalculated to show the presumed effect if the original totals equivalent had been 20 c.c. of N/20 Thio. This column is included to serve as an approximate basis for comparison of the two different experiments. The final column indicates the relative concentration of the enzyme for each inoculation, taking the concentration of the withered leaf extract used as unity.

Discussion of Results:—

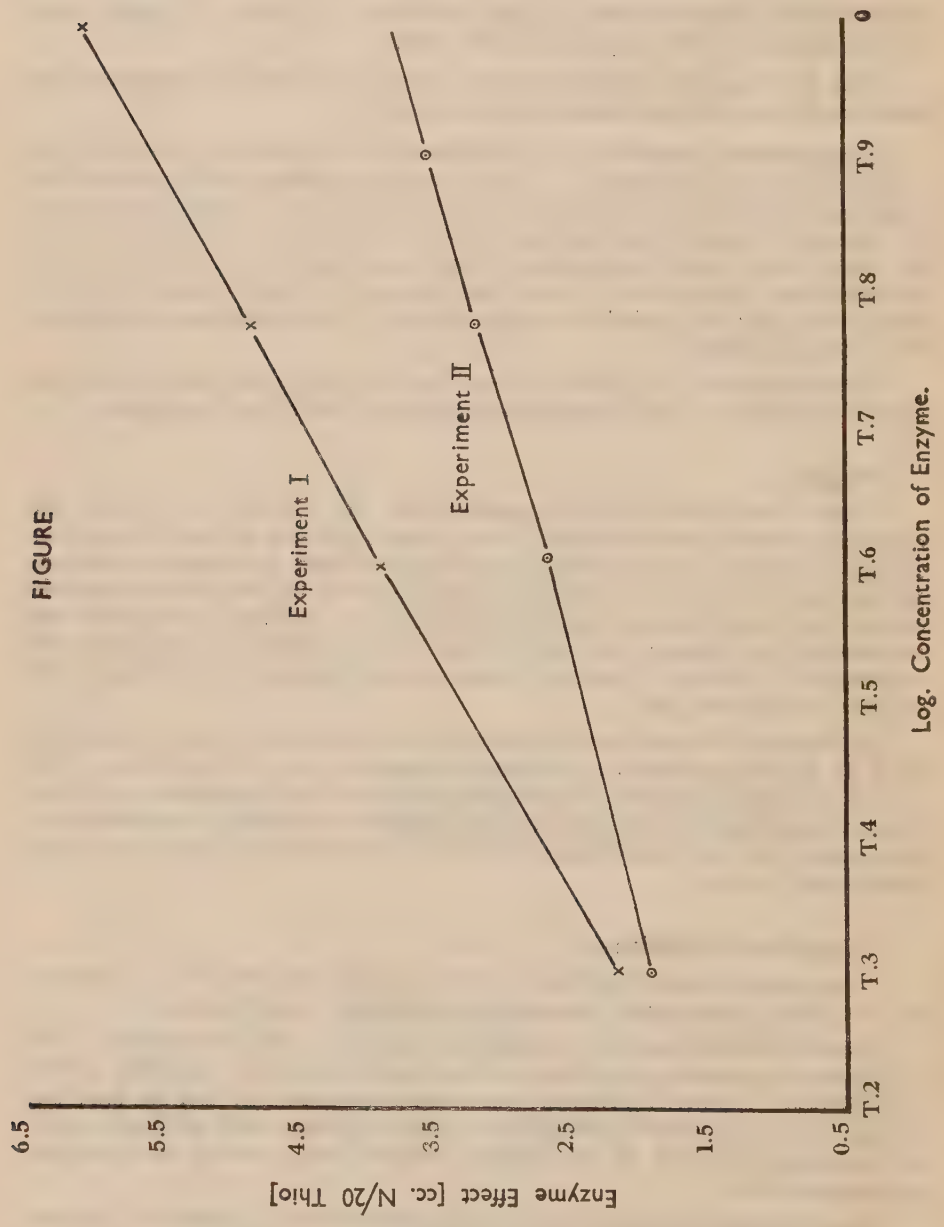
The greatest change is produced in both cases by the first 0.5 c.c. of extract, and the effect of increasing concentration diminished stage by stage.

The effect of the enzyme is a logarithmic function of the concentration. This is indicated by plotting the effect of the enzyme against the logarithm of the concentration. Figure II gives the curves thus obtained. It will be seen that in Experiment I an excellent straight line curve is obtained, while in Experiment II the smoothing required to give perfect straight line is within the limits of experimental error.

This relationship between effect and concentration is normal for enzyme action.

The results obtained are of practical use since they may be employed as a rough basis for the correction of subsequent results where a comparison is made between the effects produced by extracts of varying concentrations.

It is usual with enzyme actions for the maximum possible effect to be a constant independent of the speed with which this limit is reached.



In the two experiments discussed it is obvious that there is no likelihood of the effect in the second experiment ever becoming equal in magnitude to that observed in the first. This is probably attributable in part to a difference in the

dried leaf sample used since the method of preparing the powdered dried leaf can cause a considerable divergence in the nature of the product. By applying a hard grinding action to thoroughly dried leaf it is easily possible to reduce the bud and stalk present to an impalpable power before the true leaf fraction is reduced to the required size of flake. By more gentle treatment, and frequent removal of small fragments produced, a more representative mixture of particles, of uniform size, is obtained.

The Effect of Withering on Enzyme Activity.

Mann claimed that a marked increase in total enzyme occurred during withering. Shaw and Jones (Theotannin in Relation to Black Tea) suggested that during withering, loss of vitality resulted in a decrease in the faculty of intercellular membranes for selective permeability. It was therefore possible that theotannin might come into contact with salts of manganese or iron to form a synthetic peroxidase phase. Mann's practical results do not appear to have received any support from other investigators and there was no practical support for the suggestion made by Shaw and Jones. With the evolution of a convenient technique for the quantitative investigation of enzyme action it became possible to attempt a corroboration of Mann's results.

Experimental Procedure:—Seven 200 grams samples of withered leaf were weighed out, and spread thinly in demarcated areas to wither. At selected time intervals one sample was removed, the percentage wither estimated and an extract obtained. By regulating the amount of water added to the minced leaf before squeezing out the extract, or by adding water to the juice after extraction, the iodimetric totals value of extract used for inoculation was maintained reasonably constant. 3 c.c. were employed for the inoculation of 1.8 grams of powdered dried leaf, and a reaction time of four hours was allowed, the mean temperature of fermentation being 68° F in every case.

When designing the experiment it was anticipated that with thin spreading withering would proceed to the extent of about 60 per cent. in 24 hours, and that by taking samples at intervals of 4½ hours and using a 4-hour fermentation the experimental work could be completed in about 36 hours and cover a satisfactory range of wither and the corresponding enzyme activity.

The time chosen for the experiment unfortunately coincided with a period of exceptionally heavy and continuous rainfall with the result that the relative humidity never fell below 90 per cent., and the temperature never rose above 68° F, so that the wither was extremely protracted and the 4-hour period allotted for fermentation was too short to produce a large enzyme activity even at the maximum observed.

The results obtained are significant but should be capable of being improved by repeating the experiment under favourable conditions or with an amended procedure.

Results:—

The results obtained are given in the following table:—

TABLE X

Sample	Totals equivalent in c.c. N/20 Thio		Enzyme Effect	Percentage Wither	Duration of Wither Hrs.
	D.L. W.L.	W.L.			
A ..	18.90	0	0.00	..	..
B ..	21.95	4.0	0.95	100	0
C ..	22.50	4.5	0.90	92	4
D ..	22.95	4.85	0.80	88	8
E ..	22.40	4.4	0.90	82	12
F ..	22.10	4.5	1.30	75	16
G ..	21.15	4.45	1.85	65	48
H ..	21.10	3.6	1.40	55	72
(H) ..	..	(4.5)	(1.65)	..	..

The concentration of the extract was low in the last trial and therefore the enzyme effect is not strictly comparable with those obtained previously. The effect observed in H is practically the same as in that of F, but assuming that the dilution of H was in the ratio of 3.6 to 4.5 of that obtaining for F. the enzyme content of the leaf must have been 1.25 times that in F. Had the extract H been correctly diluted to give a totals equivalent of 4.5, the enzyme concentration would have been proportional to the logarithm of the relative concentration. A more comparable figure for the enzyme effect in H may therefore be computed as $1.4 \frac{\log 4.5}{\log 3.6}$ c.c. or 1.65 c.c.

Discussion of Results:—

Two points are obvious: (*a*) there was no increase in enzyme activity until after an 82 per cent. wither was reached, and (*b*) that after that stage there was an increased activity which was more than maintained at the end of 48 hours. Subsequently the activity of the enzyme was not greatly diminished if at all.

In this experiment no great stress can be laid on the exact changes observed in samples F, G and H. The significant points to be noted are that no change in enzyme activity occurred until after the physical wither had proceeded for over 12 hours and had reached at least 82 per cent. When the leaf became limp an increase in enzyme activity could be found..

The experiment, although imperfect, provides support for the suggestion, made by Shaw and Jones, mentioned above. Enhanced enzyme activity was only apparent when the leaf had lost some of its vitality as a result of the progress of the physical wither. It also bears out Mann's contention that there is an increase in enzyme activity as a result of withering.

Practical Significance of this Experiment:—During the last 10 years great advances have been made in the use of forced draughts of air of regulated

humidity, so that in most tea factories in S. India it is possible to obtain the required percentage of wither in a convenient time, normally about 22 hours. Hence a new technique of withering is being evolved to make the best possible use of these mechanical aids.

There is a wide diversity of opinion as to the correct duration of wither. A 22-hour wither is convenient as it can be obtained cheaply and it fits in with the remainder of the manufacturing programme. There are however extremists who claim that withers in 12 hours (or in 48 hours) give the best tea. It is therefore desirable to obtain exact information concerning the effect of duration of wither on the subsequent processes of manufacture.

The foregoing experimental work shows that the duration of wither affects the speed of fermentation. In order to maintain a standard degree of fermentation in spite of variations in speed it is necessary to make radical alteration in rolling room procedure. These changes can be worked out after a detailed study of the capacity of the machinery available, but factory managers very rarely take full cognisance of this fact. This is one reason for conflicting results arising from factory experiments on the duration of wither.

The experiment quoted promises that it is possible to work out in the laboratory, in detail, the effect of the length of wither so that authoritative generalised statements can ultimately be made governing this aspect of the question.

The Inactivation of Enzymes during the Firing of Tea.

Introduction:—The function of the firing of the fermented leaf is to stop the fermentation before it goes too far, as overfermentation leads to flat, dull, and insipid liquors.

Mann found that the enzyme he isolated was inactivated at a temperature of about 150°F and he argued that for firing to be successful the temperature of the leaf should be brought to this level as quickly as possible. He also found that if the wet leaf was exposed to a temperature of over 212°F there was a small but appreciable loss of flavour. He therefore advocated firing the tea in two processes, first putting the leaf rapidly through one machine with an air inlet temperature of not more than 220°F so that three quarters of the moisture is removed, and, secondly, returning the partly fired leaf to a second machine in which the inlet temperature is 180°F. Leaf enters a tea dryer where the air leaves the machine, and leaves the dryer near the air inlet, consequently the leaf enters the machine in both stages at a point where the air temperature is approximately 140°F.

It was realised later that it is difficult to maintain an air outlet temperature of 150°F without spoiling the tea owing to a 'case-hardening' effect. Scientists in N. E. India concluded that an inlet temperature of 180°F in the first machine was preferable although this meant a further lowering of the output of the machine. They also found that firing gave good results in practice with this inlet temperature even if the outlet temperature was as low as 120°F, from which they concluded that enzymes were inactivated at 150°F, or at 120°F in a strong blast of air.

It was found later that an outlet temperature of 120°F was not always satisfactory and that the tea was frequently 'stewed' or overfermented, and the most recent recommendation concerning firing, from the Tea Research Institute of Ceylon, is that the outlet temperature should be about 130°F, and this is probably a safe practical figure for the guidance of tea makers.

In South India and Ceylon harder withering is practised than in N. E. India and as a result firing can often be successfully carried out in one operation instead of in two.

The present writer reviewed the theory of the firing of tea in a book on 'Tea Manufacture in S. India', and concluded that the cessation of fermentation was due partly to the withdrawal of water limiting the activity of the enzyme, and partly to the action of dry heat on the enzyme itself.

Since writing this the experimental technique for investigating enzyme effects has been developed and an interesting experiment on the fall in enzyme activity during firing has been carried out.

Preliminary Investigation of Enzyme Activity in Fermented Leaf:—Before attempting to investigate the residual enzyme activity of partially fired tea it was considered advisable to find whether there was any appreciable active enzyme remaining at the end of fermentation. It was at least conceivable that the accumulation of the products of fermentation was sufficient to hinder further activity.

A quantity of withered leaf was divided into two portions, one was minced, allowed to ferment for 4 hours, water was added and the extract was squeezed out. 3 c.c. was added to 1.6 gms. of powdered, dried leaf.

The remaining withered leaf was minced, when the fermentation of the first sample was concluded, and after adding a little water the extract was obtained in the usual way. An inoculation with this extract was allowed to proceed concurrently with that of the fermented leaf extract.

Results:—

TABLE XI

	Totals equivalents in c.c. N/20 Thio	
	Initial Value	Final Value
Check Values { Withered Leaf Extract ..	3.1	2.8
Check Values { Fermented Leaf Extract ..	1.8	1.65
Dried Leaf plus Withered Leaf Extract ..	21.7	17.7
Dried Leaf plus Fermented Leaf Extract ..	20.4	19.45
Enzyme Effect of Withered Leaf ..	..	3.7
Enzyme Effect of Fermented Leaf ..	..	0.8

The fermented leaf extract was obviously less concentrated than the withered leaf extract. Comparing the concentration employed with the results obtained for Experiment I it may be concluded that by correcting for concentration the effect of the fermented leaf extract should approximately be doubled.

It is therefore assumed that the enzyme activity of fermented leaf extracts is about half that of the withered leaf extract. Against this may be set the fact that the activity of the withered leaf extract may have increased during the extra four hours withering received.

The conclusion drawn is that enzyme activity can be detected in an extract from fermented leaf but that as strong a concentration as possible is desirable in order to obtain an effect which can be measured with any accuracy.

Experiment on the Effect of Firing on Enzyme Activity:—By the courtesy of the Manager of a neighbouring estate it was possible to obtain samples of partly fired tea in which to examine the residual activity of the enzyme. For this purpose adjustments were made in the schedule of manufacture so that enough of the coarse bulk was available at one time to completely fill the dryer being used. (400 lbs. of fermented leaf). Actually there was a gap of about 2 minutes between the time at which the last of the fermented leaf was fed to the dryer intake and the time at which the fully fired tea left the machine.

As soon as fully fired coarse bulk started to come through the machine, power was shut off, the dryer was stopped for a minute or two, and the side doors of the drying chamber were opened. Samples of leaf were then drawn from the trays at points such that the periods elapsing before the leaf reached these points was known.

The samples were taken to the laboratory as soon as possible.

The moisture content of the leaf fed into the dryer was estimated, by inspection to be about 55 per cent., and it was assumed that the water content would fall smoothly to about 2 per cent. Hence the moisture content of each sample could be guessed fairly accurately. Water was added to weighed samples of the leaf according to experience gained from the data quoted in the Table. This was allowed to soak into the partially dried leaf for about 20 minutes. Excess water was then drained off, but retained. The soaked leaf was minced and the extract squeezed out. For the two driest samples of tea insufficient extract was obtainable at the first attempt and the juice drained off previously was poured over the samples and squeezed out again.

2.5 c.c. of each extract were added to 1.7 gm. of dried leaf and allowed to act for 5¾ hours at an average temperature of 75°F. 2.5 c.c. of each extract were also left for the same time at the same temperature for check values. The inoculated sets were ultimately infused and diluted to 500 c.c. in the usual way. The checks were made up to 200 c.c. only, in order to obtain a larger titration. The iodimetric totals equivalents were determined.

The moisture content of each sample of leaf was also determined.

Results:—The iodimetric results are tabulated below:—

The results quoted for sample A are not strictly comparable with those of the remaining samples since all the available coarse bulk had to be placed in the dryer in order that it should be full of one type of leaf. The figures given are derived from the most nearly comparable and available fermented leaf. The percentage moisture quoted was checked by comparing the weight of fermented leaf fed to the dryer and the weight of fired tea obtained.

The differences in enzyme effect are too small to be regarded as accurate but they show quite definitely that there is perceptible enzyme activity remaining until the leaf has been in the dryer for 10 minutes or more.

TABLE XII

Sample	Dried Leaf	Extract	Observed Totals Equivalent	Dried Leaf Totals	Enzyme Effect	Percentage Moisture in sample	Time Spent in Dryer
A ..	18.3 c.c.	2.7 c.c.	19.0 c.c.	16.3 c.c.	2.0 c.c.	56%	0 minutes
B ..	18.3 ,,	2.2 ,,	19.3 ,,	17.1 ,,	1.2 ,,	44%	$2\frac{1}{4}$,,
C ..	18.3 ,,	2.85 ,,	20.15 ,,	17.3 ,,	1.1 ,,	25%	$5\frac{1}{4}$,,
D ..	18.3 ,,	2.7 ,,	20.2 ,,	17.5 ,,	0.8 ,,	21%	$8\frac{1}{4}$,,
E ..	18.3 ,,	2.5 ,,	20.55 ,,	18.05 ,,	0.25 ,,	15%	$11\frac{1}{4}$,,
F ..	18.3 ,,	2.45 ,,	20.65 ,,	18.2 ,,	0.10 ,,	3%	$18\frac{1}{2}$,,

The variation in strength of the extract is not great enough to affect the results appreciably, except possibly for sample B, in which the extract was perceptibly weak. Probably an extract with an iodimetric value of 2.8 c.c. instead of 2.2 c.c. would have given an enzyme effect of about 1.6 or more.

The conclusion reached from this experiment is that the activity of the enzyme is reduced as the leaf being fired loses moisture but that it is not wholly inactivated until the temperature of the leaf rises, i.e. during the last stages of drying.

Many more experiments will be required in order to demonstrate optimum firing conditions from this point of view, but this single series shows that the problem can be successfully approached in this way.

It must be remembered that the enzyme activity studied here is the activity of the residual enzyme at a relatively low temperature, and not the activity which occurs in the firing machine. This must be much greater as a result of high temperature of the leaf, about 95°F to 100°F. The Figures observed are of immediate practical value since they provide concrete evidence of the danger of allowing a gap between the first and second stages of double firing when this method is employed.

It may also be mentioned that in the particular firing machine employed drying was conducted more rapidly than usual, particularly in the early stages. Consequently it may be predicted that in normal practice the extent of the enzyme action occurring in the dryer is markedly greater than that studied.

Conclusion.

The work described in this section is preliminary only. A single method has been evolved for quantitatively estimating changes in enzyme activity during the stages of tea manufacture, and some of the applications of the method have been demonstrated.

The importance of this work does not lie in the results of the individual experiments but in the developments which are possible, and the help which these developments will give in the solution of many of the problems of practical tea manufacture.

The chief practical points which it is anticipated may be cleared up by this method are as follows:—

Withering:—	Effect of rate of withering.
	Effect of temperature in the withering loft.
Fermentation:—	Effect of temperature.
	Effect of humidity.
	Effect of time.
	Effect of variations in availability of air, as influenced by ventilation and the thickness of spreading of the fermented leaf.
	Effect of particle size.
	Determination of the existence of non-enzymic fermentation, if any, under special conditions.

Firing:— Suitability of firing operations.
Effect of variation in rate of drying.
Possibility of improving the design of the dryers.

Summary.

An outline has been given of previous work on the enzyme in tea.

A method has been devised by which the action of tea enzymes on theotannin derivatives can be quantitatively estimated with accuracy. An inoculation technique is employed followed by the determination of changes in iodimetric equivalents.

The effect of time, temperature, and concentration of the enzyme have been studied. The results obtained confirm expectation and illustrate the degree of accuracy which is obtainable.

The method has been employed to determine the increase in enzyme activity as a result of withering. The results obtained confirm the practical work of Mann and theoretical suggestions made by Shaw and Jones.

The method has also been applied to determine at what stage the enzyme inactivated during the firing of tea. It was found that activity was diminished greatly before the leaf entered the firing machine but that it did not finally disappear until the drying process had been completed.

The work on this subject has only reached the preliminary stage but promises to be of great value in the solution of many problems of practical importance.

APPENDIX II

CHROMOSOME BEHAVIOUR IN THE TEA PLANT

(Camellia thea)

By M. K. Subba Rao, Mycologist.

I. Introduction.

Sir George Watt and Dr. H. H. Mann (5) observed as early as 1903 "that in a few indeed of these gardens could the jat of tea be pronounced uniform, and what was true about thirty years ago is true even today." The above remarks were made after examining some of the seed gardens that purported to be bearing pure "Indigenous" stock. Much of this impurity is probably attributable to the early gardens being established from imported "China" seed, which had opportunities of crossing with the Indigenous wild stock, resulting in numerous hybrids (natural) arising, some with desirable, and others with undesirable, characteristics.

Attempts do not appear to have been made early during the history of the industry to select the desirable members from these hybrids, at the same time destroying plants with unsuitable features. On this subject also the above mentioned authors remark "Unmistakable hybrid tea cannot infrequently be seen in some of the so-called indigenous seed gardens, but even were these removed it could hardly be said that the remainder manifested throughout a constant type." The names therefore "Assam indigenous" and "Indigenous" at the present time may only be taken to indicate members of plants possessing to a greater or less degree the characters of the parent "Indigenous" types, not always necessarily true to type.

Between the broad-leaved Assam types and the small-leaved china, numerous natural hybrids have occurred, these inheriting in various degrees the parental characters not by systematised hybridisation work but through chance. Watt and Mann have described the Assam indigenous teas as having eight primary and eight subordinate veins. The plant developing naturally grows to the dimensions of a tree 20 to 50 ft. high, rarely however with a tall stem. The leaves are dark green measuring 3 to 4″ × 1½ to 2″ with a concave upper surface forming a depression towards the midrib. The margin is sharply serrate. The plant flowers mainly on the older branches.

The "China" bush has comparatively small leaves (1 1/16–2½″ × ¾–7/16″) with not more than eight veins (four primary and four subordinate) on either side of the midrib. This plant is essentially a bush and even if given the opportunity rarely, if ever, takes the "poplar" tea form of the other races.

The "hybrids" possess from nine to fourteen pairs of veins and the leaf form shows variations from the "Indigenous" to the "China". The Manipuri has as many as sixteen pairs of veins.

Although much improvement has been made by eliminating bushes with undesirable features from seed gardens, still more requires to be done in order to keep the race of seed pure. The only reliable guarantee at present of value when seed is imported is that of the germinating capacity of the supplied seed (about 98%) without emphasis on adherence to type. The seed bearers might themselves look vigorous but their progeny, supplied in the form of seed, does not give as uniform a stand as is desired, with the result that on most gardens

mixed *jats** are the rule rather than the exception. This variation is again due to hybridisation being left to chance. Much could be done by seed garden establishments to keep their individual race of tea seed pure by systematic selection, and this method is being adopted in North-East India on some seed 'baries'.

It has been recognised in recent times that a uniform *jat* on a garden is desirable viewed either from the aspect of quality or quantity. An indifferent stand is, furthermore, a disadvantage when cultural practices have to be adopted on a large scale. To meet this problem notable contributions have been made by workers in Russia and Java. North-East India and Ceylon have also made a beginning at such an improvement. To these workers vegetative propagation, budding, grafting and inarching has afforded sufficient encouragement. The bud parents and the clones first come under observation and after selecting them work is commenced. Grafting and propagation by cuttings seem to require certain conditions not easily obtainable always, and attention is therefore directed more towards clonal selection and budding.

Such vegetatively propagated plants should however be mainly of local interest, for what might hold good for conditions in Java might not do so for Ceylon. At best this method may offer a temporary remedy because of the degeneration that might set in from continuous vegetative propagation. Another factor of importance is the economic feasibility of large scale budding or other vegetative methods employed.

Whatever the ultimate success of such methods the presence of numerous hybrids offers a large field for cytological investigations. Considerable light might be thrown by cytological investigations on the numerous natural hybrids that are of real economic value, by observing the variational behaviour in nuclear material. The results here recorded are of a preliminary nature and it is hoped to develop the chromosomal behaviour of some of the recognised Indian varieties in due course. Chromosome numbers in some of the Chinese varieties (*Camellia sinensis, Thea sinensis*) have been determined by Ono (3), Karasawa (2) and Zaldstanishvili (6).

II. Material and Methods.

Material for studying mitosis was obtained by allowing tea seeds (South Indian) to germinate on sand, the root tips being fixed after they had emerged from the hard shell. The floral material for observing meiosis was collected from a variety known generally as "Chapleton". These bushes had formed flowers during the interval between pruning and afforded a sufficient supply of material for the present investigation. Buds from two other jats ("Bonaccord"† and "China"?) have also been fixed and their chromosome numbers alone are given in this paper.

The root tips were first washed before fixing in order to remove the soil particles. At first the floral material was fixed without removing the accessory parts (calyx and the corolla) but it was found that their removal rendered the material more pliable and permitted easy penetration. Buds about 1/16 to 1/10 inch were found suitable for obtaining the several stages.

Bouin's fluid (saturated solution of Picric acid 75 c.c., Commercial Formalin 25 c.c., Glacial Acetic acid 5 c.c.), was employed to fix the root tips and the

* Jat denotes popularly a variety.

†"Bonaccord": Name of a South Indian *jat*.

flower buds. Since however the chromosomes in the mother cells of the anther tended to clump when fixed in Bouin's the material was first dipped in Carnoy's solution and immediately transferred to Bouin's and there allowed to stand for 24 hours. This method also facilitated penetration in the absence of vacuum pump to ensure the material sinking into the fixative.

The fixed material was washed in water for another period of 24 hours, dehydrated and finally embedded in the usual manner.

Sections about 8 to 10 μ thickness were found suitable for examination, these being fixed to the slide by a modified method of Mayer's formula. (5 grams of Egg albumen powder shaken with 25 c.c. of glycerine and a few crystals of sodium salicylate added to preserve the fixative). This has given very good results even after more than a year from preparation.

The sections were stained by the Hadenhain's Iron alum—Haematoxylin method and counter-stained with Orange G in clove oil.

III. Mitosis in the Root Tips.

The nucleus in the cells of the root tip occupies a central position generally and is bound by a nuclear membrane that is clearly distinguishable from the surrounding cytoplasm of the cell (Pl. IV, Fig. 46). The nucleus is spherical containing within the nuclear membrane a granular, slightly reticulate, faintly chromatic material and one or more dark staining nucleoli. A multinucleolate condition appeared to be a common feature in these cells and evidence was present suggesting the increase in number to be due to a process of budding from a single parent nucleolus. One of the nucleoli was always larger in size than the others. Two or three smaller ones, rarely four, were usual in each nucleus. Each of these nucleoli is found surrounded by a vesicular space, thus separating the nucleoli and the net work. Sharp (4) discussing the presence of the vesicular space remarks that "The broad clear zone often seen about the nucleolus in fixed material is as a general rule an artifact due to a contraction of the reticulum." Such clear zones have been found by other workers in other plants also. Dixit (1) has observed such a clear space in *Capsicum* and has cited other instances in support of this observation. From an examination of the material in tea, it seems possible that the occurrence of the clear vesicular space rather than an artifact may be characteristic of some plants.

Prophase:—The nuclear activity manifests itself by the reticulum within the nuclear membrane becoming more chromatic and resolving itself into long threads of more or less uniform dimensions (Pl. IV, Fig. 43). In the preparations obtained the continuous spireme preceding the breaking of the chromosome thread has not been observed clearly. The broken threads however show a union to have taken place along parallel lines suggesting a parasynaptic union. The multinucleolate condition of the resting nucleus and the vesicular space that surrounded the nucleolus both disappear, a single nucleolus lying freely and surrounded by the broken chromosome threads being visible. With the appearance of the chromosome threads the nucleolus loses much of its colour, becoming pale and at the same time showing a vacuolate interior.

Metaphase:—The nuclear membrane which separated the nucleus and the cytoplasm of the cell gives place to a fairly easily distinguishable zone that may be referred to as the perinuclear zone, still showing the nuclear individuality from the surrounding cytoplasm. The nucleolus also disappears and no trace of it is apparent when the chromosomes arrange themselves within the centre of the

perinuclear zone. The compact chromosome components are held by fine faintly staining long fibres running from pole to pole and giving the typical bipolar spindle (Pl. IV, Fig. 44). Not all the chromosomes touch the equatorial region, a few of these are often found placed above the equatorial region, these being generally bent.

In suitable cells as many as thirty chromosomes were counted representing the diploid (2n) number for this variety of *Camellia thea.* This number confirms the determinations of Cohen Stuart*, Zaldstanishvili (6) and Ono (3) in certain of the chinese *jats* (*Camellia sinensis* or *Thea sinensis*).

Anaphase:—The chromosomes of the metaphase spindle are pulled apart from the equatorial region and the individuals travelling along the spindle appear thinner in size. From the very few cells that were evident showing the separation of the chromosomes of the metaphase it seems likely that this stage is of a very short duration (Pl. IV, Fig. 47). The spindle is not very much pointed, but the fibres in the equator run in a parallel manner during this period.

Telophase:—On the chromosomes moving farther away from the centre and assuming a more polar position the division of the chromosomes into two groups is accomplished. This pulling apart is facilitated by the spindle fibres that connect the individual chromosomes. The chromosomes are brought closer together losing thereby much of their individuality. The spindle loses its pointed shape at the poles and a bulge again appears in the equator. At the same time a cross wall made up of granular material develops along the centre marking the initiation of the equatorial plate (Pl. IV, Fig. 48).

The chromosomes are brought more closely together during late telophase and lose all their individuality and their thread-like appearance (Pl. IV, Fig. 49). A granular outline separates the chromosome groups at either pole. The perinuclear zone is still evident but the cell plate initiated earlier is more marked in outline.

The perinuclear zone disappears after the two daughter nuclei surround themselves with a nuclear membrane and the original cell has been divided by the cross wall. The two daughter nuclei lie very near the cellplate and each has its own nuclear membrane within which the reticular network and one or more nucleoli are contained. After the cell plate has been fully organised and the nucleus possesses all its complements, the latter migrates to occupy the centre of the daughter cell and shows the characteristic appearance of a resting nucleus (Pl. IV, Fig. 50).

Tapetal Cells:—Casual examination of the tapetal cells in the anther shows practically all the stages but instead of the daughter nuclei separating from the cell and the development of a cell plate in the middle, the two nuclei are retained in the original cell itself giving it a multinucleate appearance.

IV. Meiosis (*Microsporogenesis*).

A large number of anthers placed compactly surround the pistil. In very young buds the anthers show undifferentiated layers of cells (about six) the central cells soon differentiate by taking a larger amount of stain than the ones surrounding them and develop into the pollen mother cells. The anther is four-lobed and in a transverse section each lobe shows from four to six mother cells lying freely and compactly arranged. Surrounding the mother cells but

* Cohen Stuart:—Zaldstanishvili (6) has referred to this author as having determined the somatic number in *Camellia sinensis.*

separated a short distance from them is another layer of cells, polygonal in shape and large in size constituting the tapetum. Above this are three other rows of smaller cells, and this finally constitutes the arrangement of cells in each of the pollen sacs. In longitudinal sections the same arrangement follows and the mother cells are arranged in tiers, the latter being placed periclinally.

The pollen mother cells are for a long time irregularly polygonal in shape and the individual cells are placed very close to the others imparting to each of these cells an angular appearance. All the cells however are not of uniform size or shape. This irregularity in the outline of the mother cells is maintained to the last, but the cytoplasm within condenses more and more towards the centre with the progress of the nuclear divisions. Finally however, the cytoplasm becomes spherical and floats freely within the frame work of the mother cell and the latter becomes hyaline and almost pellicular.

The Nucleus:—The nucleus of the pollen mother cell is larger in size, is independent of the differences in shape and size of the cell itself and maintains a spherical shape and almost uniform size in all of the mother cells. The nucleus is placed in a central position and is surrounded by a clear thin layer,—the nuclear membrane. Enclosed in the membrane is a reticulate, granular, very slightly chromatic net work representing the linin and one or more highly chromatic nucleoli, (Pl. I, Figs. 1–4). As in the case of the nuclei of the root tip a multinucleolate condition is common and there is clear evidence of the process of budding of the nucleoli from a single larger one. The vesicular space described earlier in the case of the vegetative nucleolus is also observed here surrounding one or more nucleoli. The nucleolus is at first highly chromatic but it pales down and becomes vacuolate as the division in the nuclear material progresses, but no regularity in chromaticity is maintained, its behaviour being somewhat erratic.

Early Prophase:—The faintly staining linin net work, as soon as the nucleus becomes active, assumes a greater amount of stain and resolves itself into a tangled mass of long granular threads (Pl. I, Figs. 5–7). It is distributed all over the nuclear space, but within the nuclear vesicle, this constitutes the so called leptotene thread. The thread is attached at various points to the single nucleolus that is at this stage visible. Where the threads crossed and intercrossed and made contact with the nucleolus, small chromatic knots appear. The intimate contact of the thread at many points with the nucleolus suggests the possibility of the former drawing some material from the latter.

Synezesis:—The *leptotene* threads form a tight knot the threads losing much of their individuality. The tight tangled knot is, furthermore, pushed towards the periphery and abuts the nuclear membrane on one of its sides. A clear space with perhaps one or two free threads obtains within the nuclear vesicle (Pl. I, Figs. 8–10). The nucleolus is situated either freely above the knot or is entangled within the mass of threads. In some of the preparations the highly chromatic nucleolus is less brilliant and clearly vacuolate even at this very early stage, but the threads almost adhere to the nucleolar structure. The nucleolus in some instances has been found to be drawn out at one of the ends and at this point the chromaticity is very great (Figs. 8–9).

The presence, or occurrence, of the synezeitic knot is considered to be an artifact due to the fixatives employed. Such a knot does not appear to be a normal feature in the unfixed nucleus. Sharp (4) has summarised the results of various investigators and comes to the conclusion that at least in some instances the occurrence of the synezeitic knot is an artifact. In the present instance this phenomenon was of common occurrence.

Zygonema. The tight knot observed during *synezesis* unravels and once again spreads within the nuclear space. The threads however have undergone considerable changes. The chromatin threads are thicker and show at certain intervals a doubled structure, union or synapsis being accomplished between parallel members is therefore parasynaptic (Pl. I, Figs. 11–14). Although the thread appears to be continuous there exist here and there regions showing a very loose contact. Also, where union between the synaptic mates has been very close the thread takes a deeper stain and gives it a glandular appearance. Such glandular regions occur throughout the thread at intervals and show by contrast the double nature of the entire thread (Pl. I, Fig. 14). The chromosome thread has set free the declining nucleolus to a large extent but has not given up contact with it. While these changes are taking place in the chromatic material of the nucleus, the nuclear membrane disappears and in its place a clear area clearly distinguishable from the surrounding cytoplasm of the cell and representing the so-called perinuclear zone becomes organised.

The cytoplasm of the mother cell is found receding from the periphery towards the centre and shows the first indications of its attempt to become rounded (Pl. I, Figs. 11–13). All the cytoplasm however has not separated from the wall, as a considerable part of it may yet be found in close contact with the polygonal wall.

Pachynema:—The double thread of the *zygotene* stage breaks up at regular intervals giving a definite number of shorter threads (Pl. II, Figs. 15–17). These chromosome threads further condense and appear to undergo a second process of pairing. The result is that the thread shows a fourfold structure giving the so-called chromosome tetrad (Pl. II, Fig. 18 a, b, c.). Each of these threads is found connected by fine, faintly staining, fibrillar strands (fibrillae) that run not only between individual portions but also between the components of each of the threads. Further shortening seems facilitated by these fine fibrillae. The union between the four components is not yet complete and uniform. When union is close, thickenings of a glandular nature are common and the chromosome itself appears knotty at intervals. With the union being complete the *diplonema* condition is presented by the chromosomes.

Diakinesis. The *diplotene* thread shortens and becomes more compact and thick, and the chromosomes bend in various ways (Pl. II, 19–22) giving the compact bivalent chromosome described generally under *diakinesis.* In suitable preparations about fifteen such thick differently shaped chromosomes are easily seen, some of the chromosomes being club-shaped, others assuming shapes of the letters S, O, X, Y, V and U, as is common in chromosomes at this stage.

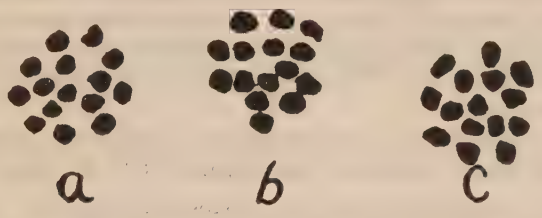
The fibrillae that made their appearance during the pachytene and diplotene stages have increased in number, at least they are more prominent than hitherto and proceed from the several members of the chromosomes forming interconnecting links.

The nucleolus is sometimes absent in the late *diakinetic* stage but most often a highly degenerated spherical mass is evident, either free (Figs. 20, 22) or in contact (Figs. 19–21) with the chromosomes.

The Heterotypic metaphase. The bivalent chromosomes straighten out and appear as short glandular chains with somewhat tapering ends. These arrange themselves with probably the aid of the fibrillae in an equatorial plane, in a lengthwise manner (Pl. II, Figs. 23–26). The bipolar spindle makes its appearance as also the "spindle fibres", these running from the chromosomes to the poles.

To a large extent the spindle is not pointed but sometimes shows an ovate appearance with its fibres also slightly curved but converging towards the poles. The fibres constituting the spindle take very little stain, although with Bouin's fixative they are supposed to take a more prominent stain, the spindle fibres comparing in this respect with the fibrillae that were described earlier. With Navaschin's fixative the fibres appeared more prominent. It is most likely that the spindle fibres originate earlier and appear in the form of fibrillae, but assuming the role of the spindle during metaphase, or, that the fibres are distinct and have a separate origin, then the fibrillae may be taken to represent the "tractile fibres" facilitating the closer association of the components of the chromosome. The arrangement of the chromosomes on the spindle is not uniform, some of the threads being apparently placed on the fibres beyond the equator, suggesting the existence of 'laggers' (Pl. II, Figs. 24–26).

From five to eight linear chromosomes are usually seen placed in a parallel manner on the spindle. In transverse median sections fifteen chromosomes arranged in a plate (Pl. II, Fig. 27) have been counted, this representing the haploid (n) number for this variety "Chapleton" of the tea plant and confirms Karasawas determination in *T. sinensis.** The haploid numbers for two other varieties viz., "Bonaccord" and "China" are the same as for "Chapleton". (Text Figs. a, b, c).



Text Fig: Chromosomes in the meiotic metaphase in the Pollen mother cells of Tea—var. *a*, "Chapleton" *b*, "Bonaccord" and *c*, "China" (?).

Karasawa (2) observed in his material trivalent chromosomes ("autotriploid") during the meiotic metaphase and also an irregular movement of the chromosomes after separation. In summarising the observations of various workers Sharp (4) has described these authors as having noticed trivalents in autotriploid plants in the meiotic prophase and during metaphase. An attempt is being made to study the occurrence of such features in the present material, but nothing conclusive has yet come to light.

The perinuclear zone demarcating the nuclear space becomes more chromatic and a granular outline surrounds this somewhat oval area. The cytoplasm has further condensed towards the centre and generally lies freely within the wall of the mother cell.

Anaphase:—The movement of the chromosomes and their separation into two groups, each drawn away from the equatorial region marks the Anaphase (Pl. III, Figs. 28–29) condition. The linear knotty chromosomes of the metaphase are now shorter and broken up and in some of the cells a certain amount of "lagging" is apparent. As the chromosomes are moved farther and farther away towards the poles the spindle shows in some instances a bulged appearance. In some of the preparations the full complement of chromosomes may be seen moving along the spindle, each group then showing fifteen individuals.

* Curr. Sci., Vol. VI, No. 9, 1938, p. 457.

Telophase:—The chromosomes having been drawn towards either of the poles, the pointed spindle is no longer seen, but the fibres are found running almost parallel from end to end (Pl. III, Fig. 30) connecting the two daughter groups of chromosomes. This, the telophasic condition marks the culmination of the 1st (heterotypic) division. The chromosomes at the poles are grouped rather closely but they still maintain their individual outline although a little over-lapping has occurred due to over-crowding.

Interkinesis:—The chromosome groups at the poles reorganise and develop a type of nuclear membrane; a nucleolus is also formed but this is smaller than the one present in the mother cell. The two daughter nuclei, although remaining within the cytoplasm of the mother cell, are separated by zones of dark staining material, which are continuous (Pl. III, Fig. 31) without any break. The individual chromosomes within the reorganised daughter nuclei are connected by fine strands of chromatic material, and the two nuclei at the poles suggest a strong resemblance to the premeiotic nucleus.

The 2nd Division:—The prophasic condition of the two daughter nuclei gives place to the 2nd metaphase spindle initiating the homotypic division of the chromosomes. The nuclear membrane and the nucleolus are disorganised and the chromosomes are arranged in an equatorial manner in two adjacent groups (Pl. III, Fig. 32). The ends of the chromosomes are attached to spindle fibres and the two spindles are slightly more pointed at the poles than their predecessor in the heterotypic metaphase. The size of the chromosomes at first appears smaller but closer examination reveals in transverse sections that they still maintain their size without reduction. The two spindles are not however as big as the 1st metaphase spindle.

In sections which have passed through the equator two groups of chromosomes, each with fifteen individuals, are apparent (Pl. III, Fig. 33).

The cytoplasm surrounding the two groups does not show any division but a granular area separates the two metaphase spindles. There is no indication of a perinuclear zone at this stage.

I. Anaphase:—The two daughter groups of chromosomes are pulled away towards the poles, and four groups of chromosomes situated on the spindle are presented. The chromosomes do not appear to move uniformly, but seem to show a somewhat uneven movement (Pl. III, Fig. 34).

Division in the two daughter groups does not proceed at the same rate,—in other words, instances of two different stages of division in the spindle were frequent. An anaphasic condition on one side and a metaphasic condition on the other side was also apparent. It is likely that this arrangement of delayed division is a prediction of the furrowing method of separation of tetrads that is to occur finally.

II. Telophase:—The telophasic arrangement wherein the four daughter groups of chromosomes have been drawn to the extremities of the poles show many peculiarities in arrangement (Pl. III, Figs. 35–36). The four groups may lie normally in a parallel adjacent manner (Fig. 36) giving four distinct groups or, one of the members of the two spindles may be placed in close proximity to the other pole giving only three distinct poles triangularly disposed (Fig. 35). The spindle shows a slight bulge in some of the preparations (Fig. 36), but the fibres straighten out and run parallel in the majority of cases (Fig. 35). The chromosomes in each group show a slight reduction in size and maintain their individuality although a certain amount of clumping is very common.

The tetrad:—The four groups of chromosomes placed at different regions in the parent cytoplasm develop their own nuclear membrane, within which they distribute themselves, and they may also be found interconnected by fine strands. The four incipient nuclei are still held by the faint spindle fibre material (Pl. III, Fig. 37). All preparations did not show the four nuclei, the majority of them showing only three of them in a given focus, the fourth however being located by changing the focal point, this tetrad arrangement being a common feature in tea as in many other dicotyledonous plants. In the meantime the four nuclei develop their nucleolar structure also (Pl. III, Fig. 38).

The separation of the four daughter nuclei each with their share of cytoplasm is accomplished by a process of furrowing from the peripheral region of the almost spherical cytoplasm of the mother cell (Pl. IV, Figs. 40–41). The individual cells of the tetrad lie superimposed one over the other and either all the four become visible at the same time (Pl. IV, Fig. 41) or only three of them are apparent (Pl. III, Fig. 39). The four cells lie floating freely within the mother cell which having given away all its cytoplasm to the daughters is more or less pellicular and hyaline in appearance. The rotation of the several members is an interesting study in material that has not been fixed.

The pollen grain:—It was not possible to obtain good preparations of pollen grains in their different stages as these tended to collapse and disperse on washing from the slides. The immature grain is slightly oval in shape and gradually assumes a spherical outline but for the three "weak spots" that are developed on the wall. The exine is smooth with no stellations. Only a single nucleus has been observed in the preparations. The wall of the grain does not appear to be uniformly thick (Pl. IV, Fig. 42).

The nucleus is fairly large with one or more nucleoli. A dark spot also appears to be common on the outside of the nucleus but what it represents is not known.

Acknowledgement:—The writer is indebted to Dr. W. S. Shaw, D.Sc., Ph.D., M.Sc., F.I.C., Chief Scientific Officer, for the kind interest evinced during the progress of the work.

Summary.

The presence of numerous natural hybrids in the tea plant suggests the possibility of a difference in chromosomal behaviour occurring in them and affords useful material for cytological studies. The present investigation deals with the mitosis in a South Indian variety of tea seed and meosis in the variety known generally as "*Chapleton*".

The nucleus in both root tip cells and the pollen mother cells occupies a central position and is bounded by a clear nuclear membrane enclosing the reticulate network and one or more nucleoli. A multinucleolate condition was a common feature.

The nucleolus was surrounded by a clear vesicle and the high chromaticity of the former seems in some way related with the nuclear activity although this did not follow any regularity. In the degenerating nucleolus, many vacuoles were noticed and the entire nucleolus disappeared just before separation of the chromosomes.

A synezeitic knot appeared always and in the case of tea it may not be an artifact. Synapsis appeared to take place between parallel members and therefore parasynaptic. The fusion between members was facilitated by the appearance of numerous "fibrillae."

Slight lagging was observed during the separation stages and a perinuclear zone appeared enclosing the spindle.

Interkinesis occurred between the heterotypic division and the 2nd division but no separation of the cytoplasm occurred. The separation of the tetrads occurred by a process of furrowing.

The pollen grain is smooth with no stellations on the exine, three "weak spots" were present and a single nucleus was observed.

The diploid (2n) number for the South Indian seed was 30 and the haploid (n) number for the varieties "Chapleton", "Bonaccord" and "China" (?) was fifteen. This probably represents the basic number.

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EXPLANATION OF PLATES

All the drawings were made with the aid of a camera lucida, Leitz 2 mm. oil immersion objective and x12 Periplan O.K. Ocular were used except Fig. 14 (I–IV) in Plate I, Fig. 18 (a, b, c) in Plate II and Fig. 45 in Plate IV for which the 2 mm. oil immersion objective and x25 periplan O.K. Ocular were employed.

Plate I.

Figs. 1 – 4 Resting nuclei in the pollen mother cells showing the budding and multinucleolate condition.

„ 5 – 7 Leptonema threads.

„ 8 –10 Synaptic contraction with nucleolus entangled.

„ 11–13 The spireme emerging from the synaptic knot in the form of loops, also shows the double nature of the thread.

„ 14 I–IV. Parasynaptic threads showing the univalents of the synaptic thread and the fusion at intervals.

Plate II.

„ 15–17 Pachynema condition with the numerous "fibrillae" running between the pachytene threads.

Fig. 18 a, b, c. The fourfold nature of the chromosomes during pachynema, also shows the chiasma and fibrillae.

„ 19–22 Diakinesis.

„ 23–26 Heterotypic metaphase, "lagging" chromosomes in Figs. 24–26.

„ 27 Chromosome plate showing the Haploid number in var. "Chapleton".

PLATE III.

„ 28–29 Heterotypic anaphase.

„ 30 Heterotypic telophase.

„ 31 Interphase showing the reorganisation of the daughter nuclei.

„ 32 Homotypic metaphase (2nd Division).

„ 33 Homotypic polar plate showing the two groups of chromosomes.

„ 34 2nd Division anaphase.

„ 35–36 2nd division telophase showing the two types of spindle arrangement.

„ 37 The tetrad showing all the four nuclei.

„ 38 The tetrad showing only three of the nuclei.

„ 39 The daughter cells of the tetrad separated.

PLATE IV.

„ 40–41 The process of furrowing in the development of the tetrad.

„ 42 The pollen grain.

„ 43–50 *Somatic divisions.*

„ 43 The broken spireme in the nucleus of the root tip.

„ 44 Metaphase spindle and chromosomes.

„ 45 Polar plate of root tip nucleus showing the diploid number of chromosomes.

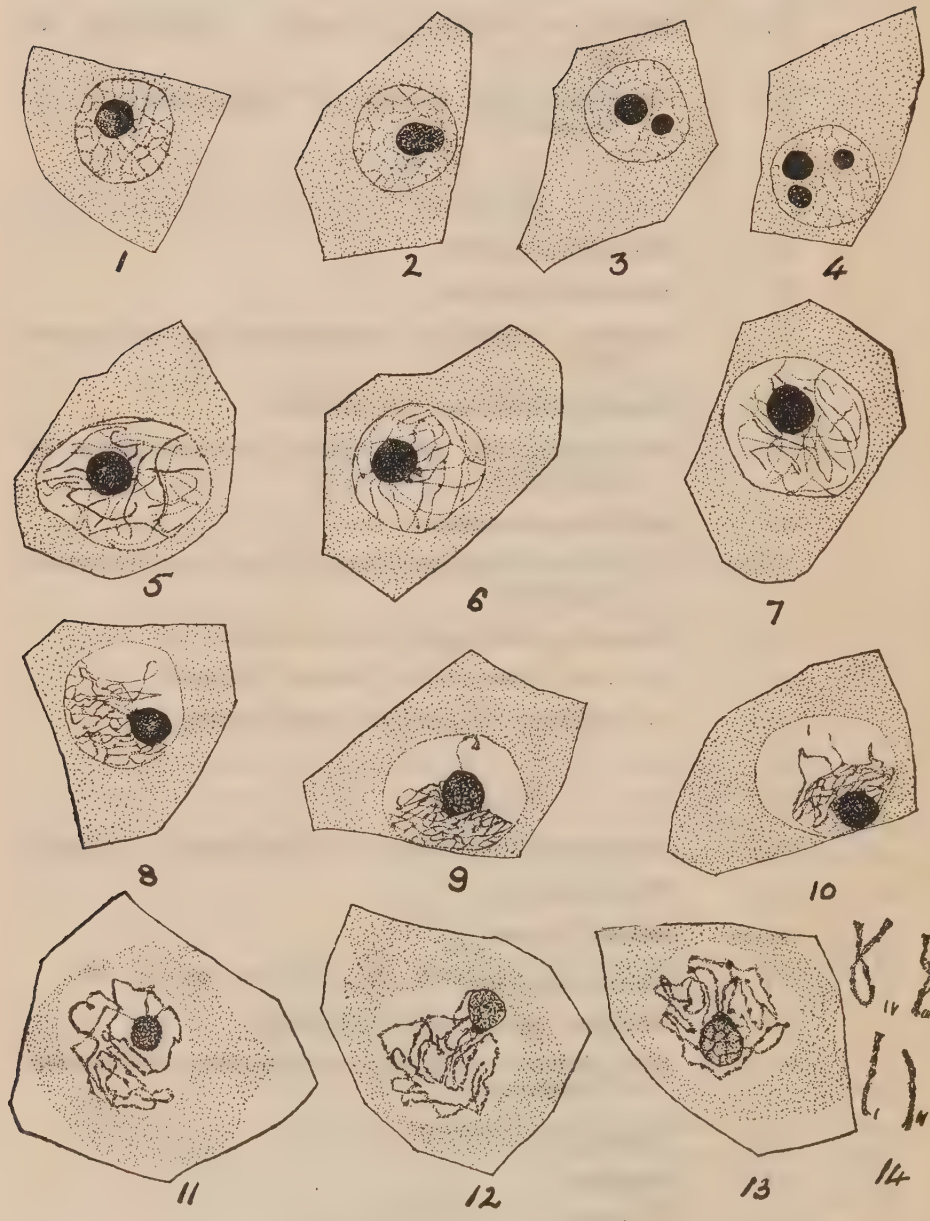
„ 46 Resting smatic nuclsai.

„ 47 Anaphase.

„ 48 Telophase.

„ 49 Late telophase showing the cell plate and the organised nuclei.

„ 50 The two daughter cells with a completed cell plate and nuclei just before separation.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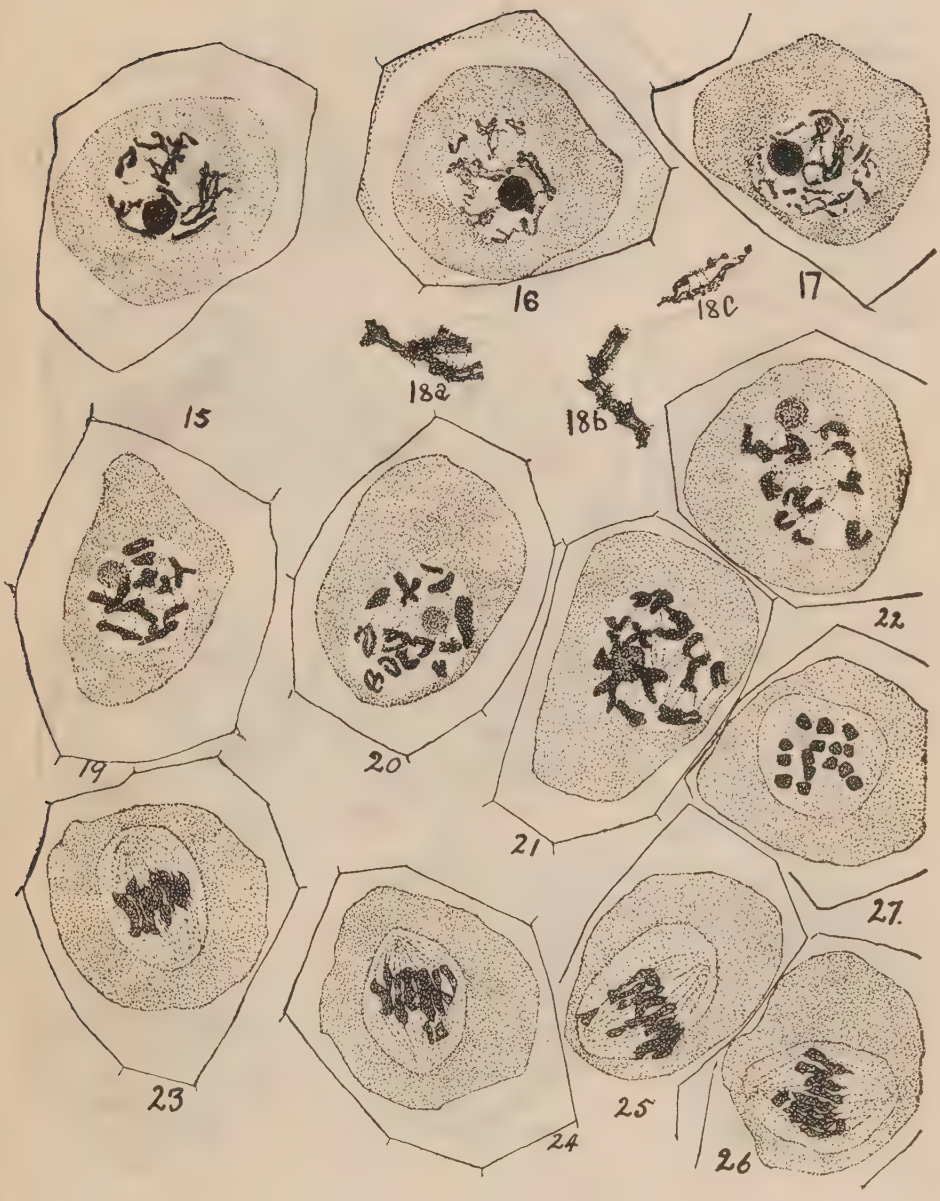
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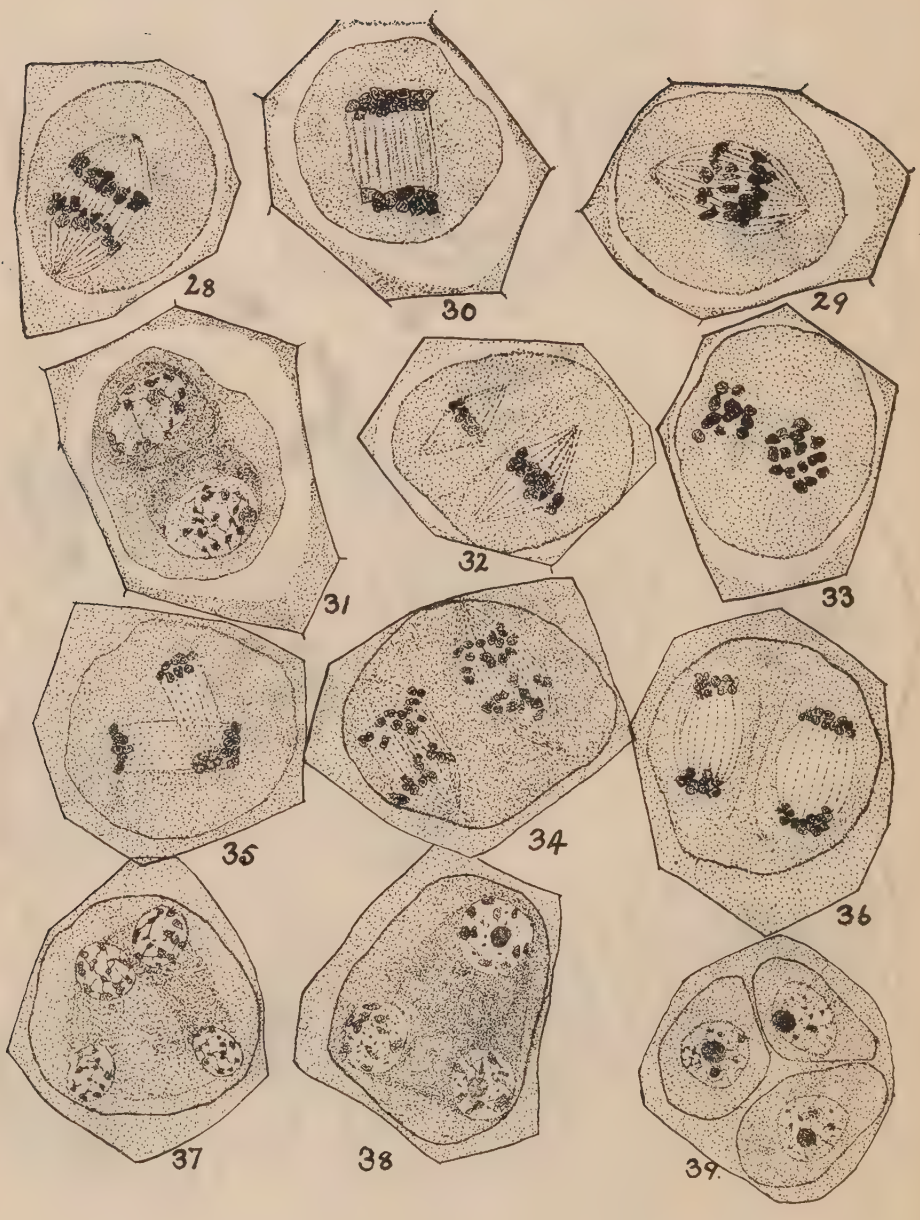
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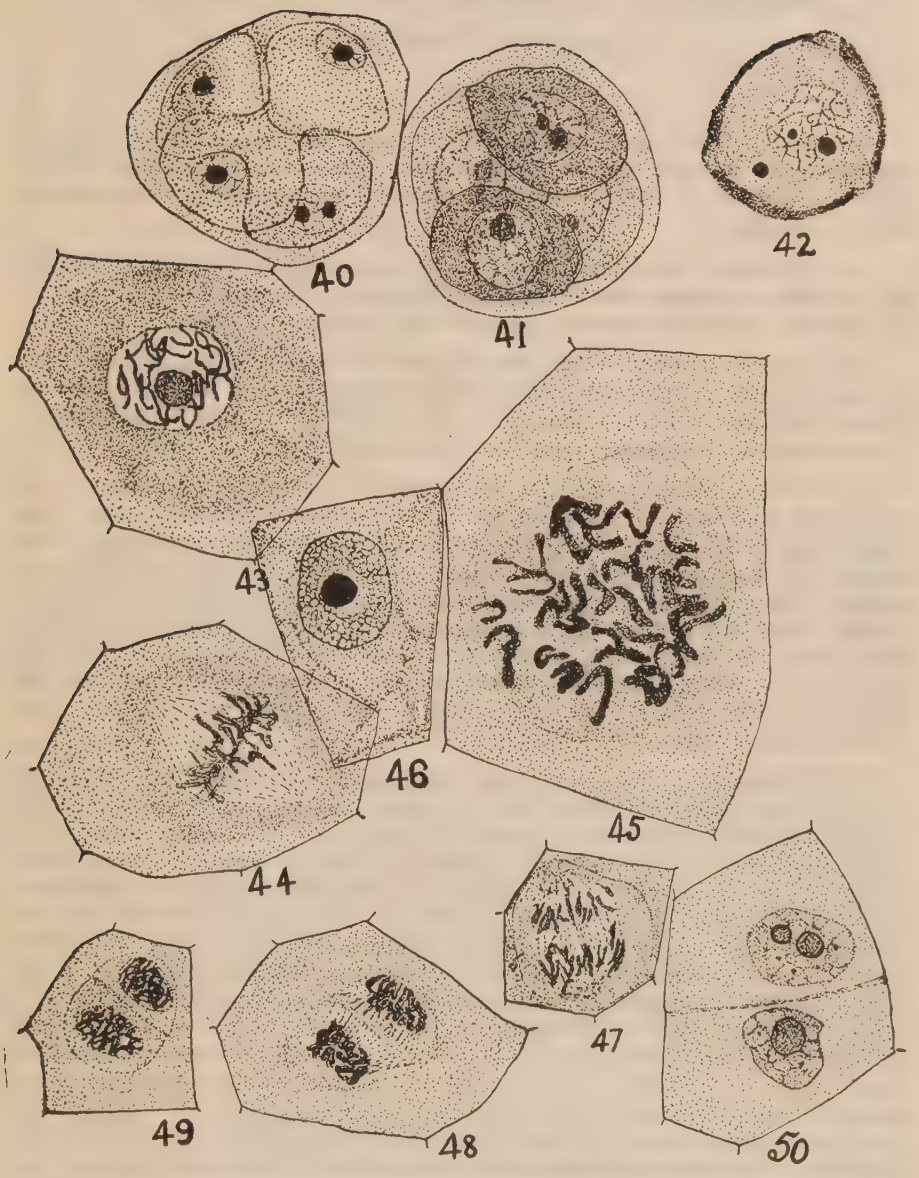
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APPENDIX III

METHODS OF COMPOST AND SOIL ANALYSIS

by V. Jayaraman, Soil Chemist.

COMPOSTS.

On arrival at the Laboratory, the moist composts are passed through a mincer and thoroughly mixed by hand. Samples reasonably dry are only mixed by hand. Stones and gravel, if any, are removed. If these are present in large amount, they are weighed.

(*a*) *Moisture*:—Moisture in 5 grams of the sample is determined by placing in a crucible in a steam oven for five hours, after which it is cooled and weighed. The decrease in weight obtained represents the 'moisture' content.

(*b*) *Loss on Ignition*:—The oven dry sample is gently heated for about ten minutes and then strongly for twenty minutes, the mass being well powdered by stirring with the flat end of a glass rod. The crucible is then cooled and weighed. The decrease in weight represents the Loss on ignition.

(*c*) *Acid insolubles*:—The ash after ignition is carefully transferred to an Erlenmeyer flask (350 c.c.) and 60 ccs. 1: 1 hydrochloric acid are added. The flask is closed by a funnel to avoid spirting and too rapid evaporation. The contents are digested for half an hour over a low flame, keeping the liquid simmering. The residue is almost white at the end of half an hour. About 60 c.c. distilled water are added and the solution filtered through a Whatman No. 4 filter paper. In the case of gelatinous residues, a Whatman No. 54 is used. The residue is washed free from hydrochloric acid (tested by silver nitrate) and the filtrate made up to 250 cc. The residue is dried, ignited and weighed. The weight obtained represents 'insolubles'.

(*d*) *Phosphate*:—100 ccs. of the hydrochloric acid filtrate are evaporated to dryness on a water-or sandbath, and the residue well heated. Keeping the dish on the hot sand, this residue is taken up again with 1 : 4 hydrochloric acid, evaporated down, and the heating continued till the last traces of hydrochloric acid have been driven off. After cooling, the phosphate is extracted with dilute nitric acid (20%). If the filtrate is not clear it is poured back through the filter paper. The residue and the sides of the filter paper are washed with dilute nitric acid two or three times. 5 grams of Ammonium nitrate are added to the filtrate and the phosphate is precipitated as ammonium phospho-molybdate by adding 20 ccs. of a mixture of equal parts of nitric acid (7 c.cs. in 3 c.cs. H_2O) and 20% ammonium molybdate. The procedure adopted is to bring the solution almost to the boil and then add the molybdate mixture (which should always be prepared fresh) by means of a pipette, stirring the contents of the beaker by a glass rod with a centrifugal motion without touching the sides. A canary yellow precipitate of ammonium phosphomolybdate is formed. It is stirred once or twice at intervals of ten minutes and then allowed to settle for two hours.

The supernatant liquid is decanted through a Whatman No. 2 filter paper retaining as much of the precipitate as possible in the beaker. The precipitate is stirred with 20 ccs. dilute nitric acid, allowed to settle and the clear liquid filtered. Further washing is done with a 3% potassium nitrate solution. After two washings the small amount of precipitate contained in the filter paper is washed back into the beaker by a strong jet of water, a fresh filter paper employed,

and filtration repeated. To test if the precipitate is free from acid, the final washings are allowed to drop into a test tube containing 1 cc. water, 1 drop sodium hydroxide (N/14), and one drop Phenolpthalin. At least 25 drops of the filtrate should be necessary to decolourise the solution. If a second filter paper is used, and about four washings employed, the precipitate is then usually free from acid. The precipitate is transferred to an Erlenmeyer flask, dissolved in 50 ccs. of caustic soda solution of known strength, boiled till free from ammonia, cooled and titrated against sulphuric acid with Phenolpthalin as indicator.

Example:—

50 ccs. NaoH=34.6 ccs. of .9376 N/10 acid.

Acid used to neutralise excess alkali=1.7 ccs.

Effective vol. of acid=34.6—1.7=32.9 ccs.

1 cc. N/10 acid=.308 Milligram of P_2O_5.

$$\%P_2O_5 \text{ (wet basis)} = \frac{100 \times 32.9 \times .9376 \times .308 \times 2.5}{48.9\%\ H_2O \qquad 1000 \times 6.651}$$

$$= .36$$

$$\% \quad \text{dry} \quad = \frac{.36 \times 100}{51.1}$$

$$= .705$$

(*e*) *Potash*:—100 ccs. of the hydrochloric acid extract (from the insolubles) are evaporated to dryness, ignited strongly till the residue is quite red, the residue being well powdered. The alkali chlorides are extracted with 50 ccs. water on a water bath, and when the volume has been reduced to about 5 ccs. it is filtered through a Whatman No. 2 filter paper into a glass dish of about 50 ccs. capacity. The residue is washed with hot water till it is free from chlorine. Any residue adhering to the dish is also well washed. The clear filtrate is concentrated to a small volume, 3 ccs. of perchloric acid (Sp. gr. 1.12) are added and the dish heated on a sand bath till dense white fumes of perchloric acid are given off. The dish is cooled, 1 cc. of perchloric acid is added and the dish is placed on the sand bath for a few minutes. After cooling, 20 ccs. of 90% alcohol are added, the precipitate stirred well and allowed to settle for 20 minutes when the supernatant liquid is decanted through a Whatman No. 2 filter paper. As much as possible of the precipitate is retained in the dish. The precipitate is then washed with alcohol containing 1 cc. perchloric acid per 100 ccs. of alcohol, 4 to 6 times by decantation. A final washing is given with 95% alcohol to remove the last traces of acid. The precipitate is dissolved in hot water, the filter paper also being well washed. The resulting solution is evaporated to dryness, the dish heated in an air oven at 135°C. for one hour, cooled and weighed.

Example:—

Weight of the Potassium Perchlorate= .124 gm.

1 gm. of Potassium Perchlorate= .3399 gm. of Potassium oxide (K_2O).

$$\%\ K_2O \text{ (wet basis)} = \frac{.124 \times .3399 \times 2.5 \times 100}{48.9\%\ H_2O \qquad 6.651}$$

$$= 3.1$$

$$\% \quad \text{„} \quad \text{Dry basis} = \frac{1.585 \times 100}{51.1}$$

$$= 1.585$$

(*f*) *Nitrogen*:—About 3 grams of the compost, accurately weighed, are transferred to a Kjeldhal flask, 20 ccs. of water added followed by 23–25 ccs. of concentrated sulphuric acid. The flask is gently heated till frothing ceases and 7 grams of Potassium bisulphate and a crystal of copper sulphate are added. A small funnel is placed on the mouth of the flask which is then heated strongly, the digestion continuing till the black colour changes to straw yellow or pale green. After cooling, the contents are transferred to a distillation flask (containing glass beads to insure steady boiling) with water, retaining as much of the insoluble portion as possible in the flask, and the solution boiled, the liberated ammonia being received, via an adapter, into 20 ccs. of standard sulphuric acid containing methyl red as an indicator. Distillation is continued till 250 to 300 ccs. of the distillate are collected. The end of the adapter is maintained just under the surface of the distillate. The distillate is finally transferred to a flask, boiled for five minutes, cooled and titrated against N/14 NaoH solution. The difference between the original 20 ccs. acid and final titration expressed as ccs. N/14 caustic soda, multiplied by 1.0 gives the weight of nitrogen in milligrams in the weight of sample taken. The percentage nitrogen may then be calculated.

Preparation of Sulphuric acid solution:—About 2.7 ccs. of conc. acid in 1000 ccs. of water gives approximately N/10 solution. 100 ccs. of the solution are pipetted into a 400 cc. beaker. In another beaker 1.5 grams of Barium chloride are dissolved in 100 ccs. of distilled water. A pinch of ammonium chloride is added, followed by two or three drops of hydrochloric acid. The two solutions are brought to the boil separately, and the barium chloride solution added to the acid solution slowly. The precipitate of Barium sulphate is allowed to settle for 8 hours, filtered, washed free from chloride with warm water, transferred to a weighed crucible, dried, ignited, cooled and weighed. From this weight the normality of the acid and also of the alkali are calculated as follows:—

Weight of $BaSo_4$ ppt. $= 1.350$ grams.

$\therefore$ Normality of acid $= \frac{1.350}{1.167} = 1.157$ N/10.

20 ccs. of the acid are titrated against the caustic soda solution and the normality of the caustic soda solution obtained by calculation:—

20 ccs. acid $= 31.7$ ccs. of alkali.

$\therefore$ Normality of alkali $= \frac{20 \times 1.157}{31.7} = .07298$ N.

(*g*) Carbon:—0.2 grams of compost are taken in a test tube. In the case of dry composts a smaller quantity is employed. 20 ccs. of chromic acid solution are added. This solution is prepared by dissolving 16 gms. of chromic acid in 1000 ccs. of conc. sulphuric acid and heating the mixture at 165°C. for half an hour. The compost is mixed well with the solution by vigorous shaking. The oxidation of organic matter is brought about by inserting the test tube in a sulphuric acid bath maintained at 165°C. for ten minutes. A blank with 20 ccs. chromic acid solution is also inserted. The test tubes are then taken out and allowed to cool. Meanwhile 100 ccs. of recently boiled and cooled water are taken in a 600 cc. flask and 10 ccs. of 85% phosphoric acid solution are added. The contents of the test tube after cooling are transferred to the flask with 150 ccs. of boiled and cooled water. 10 drops of Diphenylamine solution (prepared by dissolving .5 grams in 100 ccs. of concentrated sulphuric acid and 20 ccs. of water) are added. The indicator turns the solution deep blue. The solution

is titrated with Ferrous Ammonium sulphate prepared by dissolving 79.5 grams. in 1000 ccs. of water containing 40 ccs. conc. sulphuric acid. The titration is continued to the total disappearance of blue from the green solution i.e. till the solution turns muddy green. (15–16 samples may be oxidised in the same sulphuric acid bath).

Example:—

1 cc. of Ferrous Ammonium sulphate = .0005955 gm. of carbon.
Weight of compost = .200 gm.
Volume of Ferrous Ammonium sulphate = 9.0 cc.
„ „ for blank = 38.0 cc.
∴ Effective volume = 29.0 ccs.

$$\% \text{ carbon (wet basis)} = \frac{29 \times .0005955 \times 100}{.200}$$

48.9% H_2O

$$= 8.6$$

„ dry

$$= \frac{8.6 \times 100}{51.1}$$

$$= 16.8$$

ANALYSIS OF SOILS

1. Chemical.

The sample of soil is air dried for two days. Organic matter such as foot hairs etc., is carefully removed. Large clumps of soil are powdered by means of a wooden pestle. Gravel and stones are removed by passing the soil through a 2 mm. sieve and weighed.

(*a*) *Moisture*:—20 grams of the fine earth are used for the moisture determination, the loss in weight after five hours in the steam oven representing the moisture content.

(*b*) *Loss on Ignition*:—The oven dry soil is gently heated on a low flame for about 10 minutes and then strongly for 20–30 minutes to determine the loss on ignition. During ignition the soil is well stirred. It is cooled and weighed.

(*c*) *Acid insolubles*:—Digestion with hydrochloric acid is carried out by transferring ignited soil to a flask, adding 40 ccs. conc. hydrochloric acid and placing the flask for eight hours on a hot sand bath. The mouth of the flask is closed with a funnel to avoid spirting and too rapid evaporation. At the end of eight hours the flask is cooled, contents filtered through a Whatman No. 4 filter paper into a 500 cc. graduated flask, and the residue washed with hot water till free from chlorine. The residue is dried, ignited and weighed. It represents Insoluble silicious matter. The filtrate is made up to 500 ccs.

(*d*) *Carbon*:—0.5 gram of the sample is weighed out and transferred to a test tube. 20 ccs. of chromic acid mixture are added and during digestion (about 15 minutes) the contents are mixed well by frequent stirring with a glass rod kept in the test tube. While washing the contents into the flask after cooling, the glass rod is also washed well. The procedure resembles the estimation of carbon in composts. The percentage organic matter may be determined by multiplying the 'carbon' percentage by 1.72 (this figure requires confirmation).

(*e*) *Nitrogen*:—Nitrogen is estimated in 3–5 grams of the soil as in the case of composts.

(*f*) *Total Phosphate*:—150 ccs. of the hydrochloric acid extract are evaporated to dryness in a dish, which is then heated for two hours at 120°C. in an air oven to dehydrate silica. The dish is then cooled, the residue taken up with 20 ccs. 1 : 4 hydrochloric acid, evaporated again to dryness, powdered well and heated on the sand bath till the last traces of hydrochloric acid are driven off. Dilute (20%) nitric acid is added and the silica allowed to settle for some hours. It is then filtered, washed with dilute nitric acid two or three times, and then with water. Phosphate estimation is carried out in the filtrate as already detailed under 'composts'.

(*g*) *Total Potash*:—150 ccs. of the hydrochloric acid are evaporated to dryness, ignited till the residue is quite red, the dry mass being well powdered. The residue is digested with 50 ccs. water on a water bath and when the volume has been reduced to about 5 ccs. it is filtered, and washed well with hot water till free from chlorine. Potash estimation is then carried out as detailed under 'Composts'.

Available Plant Foods.

A weight of air dry soil equal to 200 grams of dry soil is shaken with 2000 ccs. of 1% citric acid solution in a Winchester bottle for 7 days, at regular intervals each day. At the end of this period the liquid is allowed to settle overnight. The clear supernatant liquid is siphoned off.

(*h*) *Available Phosphate*:—500 ccs. of the citric acid extract, equal to 50 grams of dry soil, are concentrated to a small bulk in a 800 cc. beaker, transferred to a dish, evaporated to dryness, ignited at a low temperature to drive off organic matter and citric acid, the mass being well powdered. The residue is treated with 30 ccs. of 1 : 1 hydrochloric acid, evaporated to dryness and the dish heated in an oven at 120°C for 2 hours. The residue is then extracted with dilute nitric acid and estimation of phosphate is carried out as usual.

(*i*) *Available Potash*:—500 ccs. of the citric acid extract are concentrated to a small bulk in a 800 cc. beaker, transferred to a dish, evaporated to dryness, ignited at a low temperature, residue treated with 30 ccs. 1 : 1 hydrochloric acid, evaporated to dryness, gently ignited and then extracted with water and the potash determined in the usual way.

(*j*) *Iron and Alumina*:—150 ccs. of the extract are transferred to a 400 cc. beaker, about 1 gram of solid ammonium chloride is added and the solution boiled. Ammonia is added, the solution being stirred continuously, till the escaping steam smells distinctly of ammonia. Boiling is continued for a minute or two. The flocculent precipitate is allowed to settle, and the supernatant liquid decanted through a Whatman No. 2 filter paper, washing of the precipitate being done by decantation with hot water. The precipitate is then transferred to the filter, traces adhering to the sides of the beaker being removed by means of a policeman. Washing is continued till the washings are free from chlorine, after which the precipitate is dried in the steam oven. It is then carefully removed from the filter paper to a porcelain dish. The filter paper alone is ignited to ash in a weighed crucible carefully warmed to expel the acid and then heated strongly. The main bulk of the precipitate is now added to the crucible which is heated for 10 minutes, cooled and weighed. The weight of the residue represents the sum of the iron and aluminium oxides.

(*k*) *Iron*:—The remaining 50 ccs. of the hydrochloric acid extract are evaporated to dryness in a dish. The residue is taken up with 40–50 ccs. dilute

sulphuric acid and transferred to an Erlenmeyer flask. A few zinc clippings free from iron are added. The solution is warmed to start the reaction. The mouth of the flask is covered by a funnel (preferably with a Bunsen valve). The completion of reduction is tested by removing a drop of the solution with a glass rod and allowing it to react with a drop of Potassium thiocyanate solution on a white porcelain dish. The contents are filtered, and residue washed with hot water. To the filtrate, dilute sulphuric acid is added and the solution titrated against N/10 Potassium permanganate till a faint pink colour persists.

1 cc. N/10 $KMnO_4$ = .0072 gram of Ferrous oxide (FeO).
= .008 gm. Ferric oxide (Fe_2O_3).

The difference between percentage of Fe_2O_3 + Al_2O_3, and FeO_3 alone, gives percentage of Al_2O_3.

(*l*) *Lime*:—The filtrate from the estimation of Iron and Alumina is concentrated to a small bulk. A gram of solid ammonium chloride is added followed by ammonia till the solution smells of ammonia. The solution is then heated to boiling and 2 grams of freshly powdered Ammonium oxalate are added slowly, stirring continuously. Boiling is continued for about two minutes and the precipitate allowed to settle. Complete precipitation is tested by adding carefully down the sides of the beaker a small quantity of Ammonium oxalate solution and examining whether any further precipitate is formed. The supernatant liquid is filtered through a Whatman No. 42 filter paper, and the precipitate washed with warm water till free from chloride. A hole is then made in the filter paper and the precipitate washed down into a flask with warm water and finally with warm dilute sulphuric acid. The acid is poured on the sides of the filter paper after opening the folds. Dilute sulphuric acid is added in excess, the solution heated to 70°C., and titrated against N/10 Potassium permanganate till a faint pink colour persists.

1 cc. N/10 $KMnO_4$ = .0028 gm. of CaO.

Standardisation of Potassium permanganate solution:—

.63 gram of oxalic acid in 100 ccs. water gives N/10 solution. 3.19 grams of permanganate in a litre of water will give an N/10 solution.

10 ccs. of the oxalic acid solution are transferred to a flask, 20–30 ccs. dilute sulphuric acid are added, heated to 70°C and titrated against the permanganate while hot. The first few drops are added slowly noting whether the colour due to the previous drop has disappeared. Afterwards titration is carried on as usual till a faint pink colour persists.

(*m*) *Magnesium*:—Magnesium is determined in the filtrate from the estimation of calcium. The filtrate is concentrated to a small bulk and transferred to a weighed dish and evaporated to dryness. To prevent the salt from creeping over, white vaseline is smeared around the top of the dish. When the residue is completely dry it is ignited till no further white fumes are evolved and the residue is white. It is cooled and weighed. The weight of the residue gives the combined weight of magnesium chloride + potassium chloride + sodium chloride. Ammonium salts volatalise and magnesium chloride is converted to oxide during ignition. The residue is taken up with dilute hydrochloric acid, transferred to a beaker, rendered alkaline with ammonia and magnesium precipitated with sodium phosphate and magnesium ammonium phosphate. The precipitate is washed with 1 : 3 Ammonia, dried, ignited and weighed as magnesium pyrophosphate from which percentage of magnesia is calculated.

(*n*) *Soda*:—The percentages are known of

(1) Magnesium oxide + Potassium chloride and Sodium chloride.

(2) Magnesium oxide alone

and (3) Potash alone.

Hence percentage of soda can be obtained by difference.

2. **Mechanical**:—

Mechanical analysis on the portion passing through the 2 mm. sieve (see 'Chemical') is done as follows:—

(*i*) *Coarse sand*:—20 grams of the earth are gently boiled over a low flame with 100 ccs. of water in a 400 cc. beaker till the volume is reduced to about 20 ccs. After cooling, the contents are poured on to a 70 mm. sieve thoroughly wetted placed over a large dish. Coarse sand is retained by the sieve. It is washed four or five times till the washings run clear and contain no fine sand. Clayey soils require more washing than sandy soils. The coarse sand is transferred to a weighed dish, dried and weighed.

(*ii*) *Silt and Clay*:—The muddy liquid in the china dish consisting of fine sand, silt and clay is transferred to a 600 cc. beaker, volume made up to 500 cc. and 4 cc. of N. caustic soda are added. The liquid is well stirred at regular intervals for two hours and then transferred to a 1000 cc. cylinder, another 4 ccs. of N. caustic soda added and the volume made up to the 1000 cc. mark. The cylinder is covered tightly with a glass disc and shaken thoroughly end over end for one minute. The time is now noted and at the end of 4 minutes 48 seconds 'silt + clay' sampling is carried out by removing 20 cc. of the liquid at a depth of 10 cms. by means of a Robinson's pipette held in position by a cork wide enough to cover the mouth of the cylinder. The pipette is lowered into the liquid slowly about 20 seconds before the time allotted and at the end of four minutes 48 seconds the liquid is sucked up slowly avoiding too rapid an ingress of the solution into the pipette. (The 10 cm. mark is made on the stem of the pipette). The time permitted for settling varies according to the temperature. The 20 ccs. are delivered into a weighed china dish, evaporated to dryness, dried and weighed. The weight of 'silt + clay' portion in milligrams divided by four gives the percentage of 'silt + clay'.

The cylinder is shaken again thoroughly for one minute and the contents allowed to settle for 8 hours when the clay sampling is carried out at a depth of 10 cms. with a Robinson's pipette. The 20 ccs. are delivered into a weighed china dish, dried and weighed. The weight of clay in milligrams divided by four gives the percentage of 'clay'.

(*iii*) *Fine sand*:—Major portion of the liquid in the cylinder is now thrown away and the sediment at the bottom is transferred to a small 100 ccs. cylinder with a mark at the side at a height of 10 cm. from the bottom, making allowance for the probable thickness of the sediment. Water is added to the mark, stirred well for a minute, allowed to settle for 4 minutes 48 seconds, when the supernatant liquid is decanted. This process is repeated till the supernatant liquid is quite clear. The fine sand fraction which remains at the bottom is transferred to a weighed dish, dried and weighed.

The results are tabulated as moisture, coarse sand, fine sand, silt and clay.

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